(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
22 November 2001 (22.11.2001)

PCT

(10) International Publication Number
WO 01/87895 A1

(51) International Patent Classification[7]: C07D 515/22, 491/22, 471/18, A61K 35/00 // (C07D 515/22, 317:00, 291:00, 241:00, 221:00, 221:00) (C07D 491/22, 317:00, 241:00, 221:00, 221:00) (C07D 471/18, 241:00, 221:00, 221:00)

(21) International Application Number: PCT/GB01/02120

(22) International Filing Date: 15 May 2001 (15.05.2001)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
PCT/GB00/01852 15 May 2000 (15.05.2000) GB

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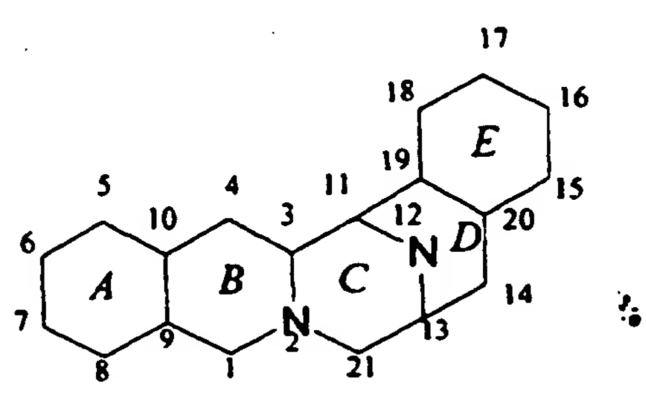
(81) Designated States (*national*): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.

(84) Designated States (*regional*): ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published:
— *with international search report*
— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: SYNTHETIC PROCESS FOR THE MANUFACTURE OF AN ECTEINASCHIDIN COMPOUND



(XIV)

(57) Abstract: Processes are provided for preparing compounds with a fused ring structure of formula (XIV). Such products include ecteinascidins and have a spiroamine-1,4-bridge. The process involving forming a 1,4 bridge using a 1-labile, 10-hydroxy, 18-protected hydroxy, di-6,8-en-5-one fused ring compound. After formation of the 1,4 bridge, C-18 protection is removed before spiroamine introduction.

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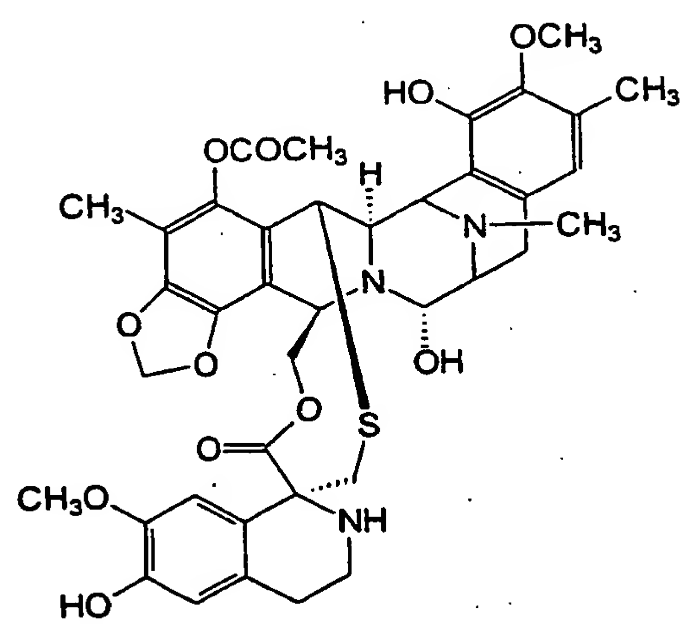
SYNTHETIC PROCESS FOR THE MANUFACTURE OF AN ECTEINASCHIDIN COMPOUND

The present invention relates to synthetic processes, and in particular it relates to synthetic processes for producing ecteinascidin compounds.

BACKGROUND OF THE INVENTION

European Patent 309,477 relates to ecteinascidins 729, 743, 745, 759A, 759B and 770. The ecteinascidin compounds are disclosed to have antibacterial and other useful properties. Ecteinascidin 743 is now undergoing clinical trials as an antitumour agent.

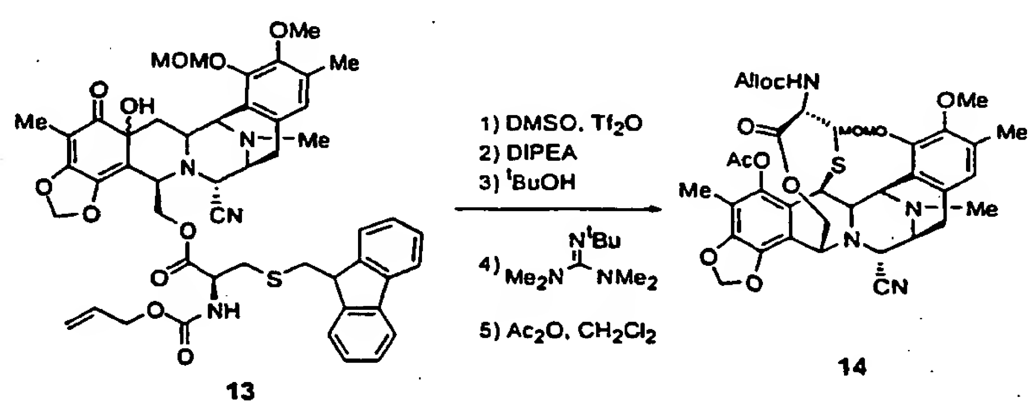
Ecteinascidin 743 has a complex tris(tetrahydroisoquinolinephenol) structure of the following formula (I):



It is currently prepared by isolation from extracts of the marine tunicate *Ecteinascidin turbinata*. The yield is low, and alternative preparative processes have been sought.

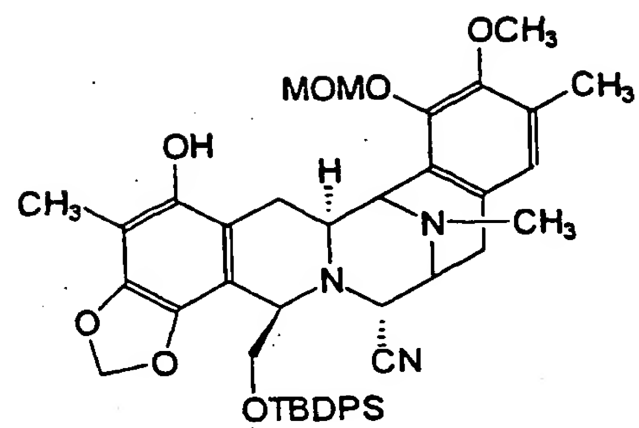
A synthetic process for producing ecteinascidin compounds is described in US Patent 5,721,362, see also WO 9812198 which is incorporated herein by reference in full. The claimed method is long and complicated, there being 38 Examples each describing one or more steps in the synthetic sequence to arrive at ecteinascidin 743.

In the known synthetic process, a 1,4 bridge is formed using a 1-labile, 10-hydroxy, 18-protected hydroxy, di-6,8-en-5-one fused ring compound. As shown in Example 33, a compound (13) is converted to compound (14):



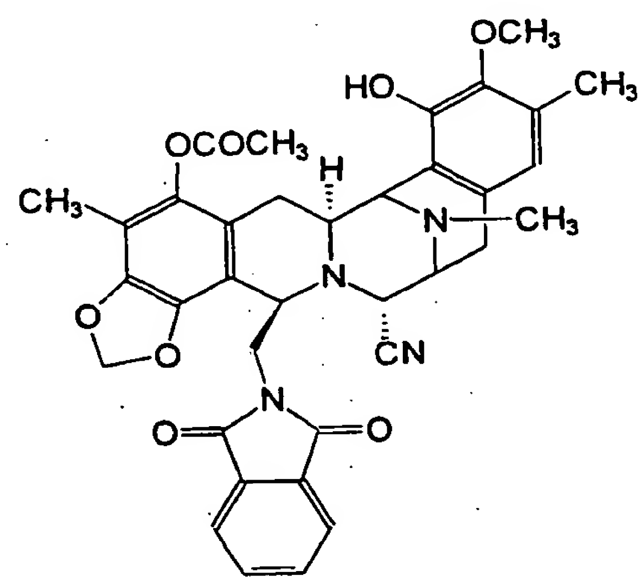
According to the known synthetic process, a spiroquinoline is then formed in the 1,4 bridge by the steps of Examples 34 to 36, and the 18-MOM protecting group is removed to give ecteinascidin 770 which can then be converted to ecteinascidin 743.

Claim 25 of US 5,721,362 is directed at an intermediate phenol compound of a given formula (11), which we refer to also as Intermediate 11 or Int-11. It has the following bis(tetrahydroisoquinolinephenol) structure (II):

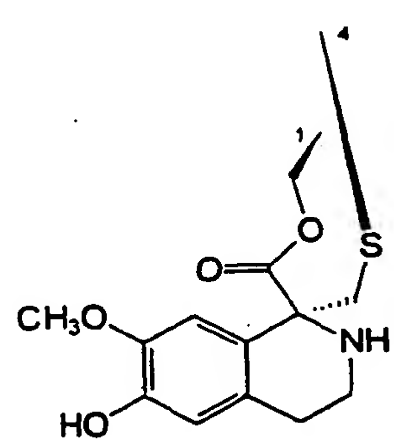


where MOM is a methoxymethyl substituent and TBDPS is a tert-butyldiphenylsilyl substituent.

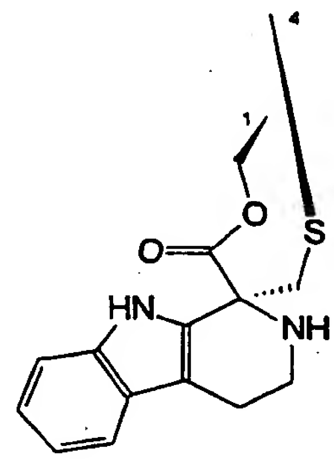
From Intermediate 11 it is possible to synthesise another interesting antitumour agent, phthalascidin, see Proc. Natl. Acad. Sci. USA, 96, 3496-3501, 1999. Phthalascidin is a bis(tetrahydroisoquinolinephenol) derivative of formula (III):



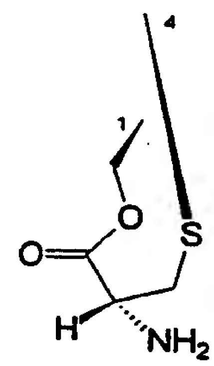
In ecteinascidins 743 and 770, the 1,4 bridge has the structure of formula (IV):



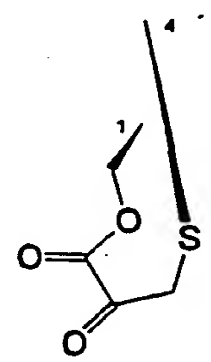
Other known ecteinascidins include compounds with a different bridged cyclic ring system, such as occurs in ecteinascidin 722 and 736, where the bridge has the structure of formula (V):



ecteinascidins 583 and 597, where the bridge has the structure of formula (VI):



and ecteinascidin 594 and 596, where the bridge has the structure of formula (VII):



The complete structure for these and related compounds is given in J. Am. Chem. Soc. (1996) 118, 9017-9023. This article is incorporated by reference.

Other literature on the ecteinasdin compounds includes: Corey, E.J., *J. Am. Chem. Soc.*, 1996, 118 pp. 9202-9203; Rinehart, et al., *Journal of Natural Products*, 1990, "Bioactive Compounds from Aquatic

and Terrestrial Sources", vol. 53, pp. 771-792; Rinehart et al., *Pure and Appl. Chem.*, 1990, "Biologically active natural products", vol 62, pp. 1277-1280; Rinehart, et al., *J. Org. Chem.*, 1990, "Ecteinascidins 729, 743, 745, 759A, 759B, and 770: potent Antitumour Agents from the Caribbean Tunicate *Ecteinascidia turninata*", vol. 55, pp. 4512-4515; Wright et al., *J. Org. Chem.*, 1990, "Antitumour Tetrahydroisoquinoline Alkaloids from the Colonial ascidian *Ecteinascidia turbinata*", vol. 55, pp. 4508-4512; Sakai et al., *Proc. Natl. Acad. Sci.* USA 1992, "Additional anitumor ecteinascidins from a Caribbean tunicate: Crystal structures and activities *in vivo*", vol. 89, 11456-11460; *Science* 1994, "Chemical Prospectors Scour the Seas for Promising Drugs", vol. 266, pp.1324; Koenig, K.E., "Asymmetric Synthesis", ed. Morrison, Academic Press, Inc., Orlando, FL, vol. 5, 1985, p. 71; Barton, et al., *J. Chem Soc. Perkin Trans.*, 1, 1982, "Synthesis and Properties of a Series of Sterically Hindered Guanidine bases", pp. 2085; Fukuyama et al., *J. Am. Chem. Soc.*, 1982, "Stereocontrolled Total Synthesis of (+)-Saframycin B", vol. 104, pp. 4957; Fukuyama et al., *J. Am. Chem. Soc.*, 1990, "Total Synthesis of (+) – Saframycin A", vol. 112, p. 3712; Saito, et al., *J. Org. Chem.*, 1989, "Synthesis of Saframycins. Preparation of a Key tricyclic Lactam Intermediate to Saframycin A", vol. 54, 5391; Still, et al., *J Org. Chem.*, 1978, "Rapid Chromatographic Technique for Preparative Separations with Moderate Resolution", vol. 43, p. 2923; Kofron, W.G.; Baclawski, L.M., *J. Org. Chem.*, 1976, vol. 41, 1879; Guan et al., *J. Biomolec. Struc. & Dynam.*, vol. 10, pp. 793-817 (1993); Shamma et al., "Carbon-13 NMR Shift Assignments of Amines and Alkaloids", p. 206 (1979); Lown et al., *Biochemistry*, 21, 419-428 (1982); Zmijewski et al., *Chem. Biol. Interactions*, 52, 361-375 (1985); Ito, *CRC Crit. Rev. Anal. Chem.*, 17, 65-143 (1986); Rinehart et al., "Topics in Pharmaceutical Sciences 1989", pp. 613-626, D. D. Breimer, D. J. A. Cromwelin, K. K. Midha, Eds., Amsterdam Medical Press B. V., Noordwijk, The Netherlands (1989); Rinehart et al., "Biological Mass Spectrometry", 233-258 eds. Burlingame et al., Elsevier Amsterdam (1990); Guan et al.,

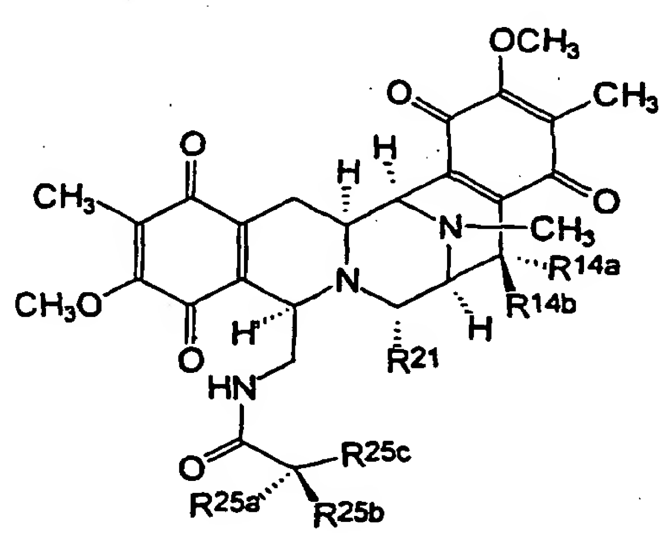
Jour. Biomolec. Struct. & Dynam., vol. 10 pp. 793-817 (1993); Nakagawa et al., *J. Am. Chem. Soc.*, 111: 2721-2722 (1989);; Lichter et al., "Food and Drugs from the Sea Proceedings" (1972), Marine Technology Society, Washington, D.C. 1973, 117-127; Sakai et al., *J. Am. Chem. Soc.*, 1996, 118, 9017; García-Rocha et al., *Brit. J. Cancer*, 1996, 73: 875-883; and Pommier et al., *Biochemistry*, 1996, 35: 13303-13309.

Further compounds are known which lack a bridged cyclic ring system. They include the bis(tetrahydroisoquinolinequinone) antitumor-antimicrobial antibiotics safracins and saframycins, and the marine natural products renieramicins and xestomycin isolated from cultured microbes or sponges. They all have a common dimeric tetrahydroisoquinoline carbon framework. These compounds can be classified into four types, types I to IV, with respect to the oxidation pattern of the aromatic rings.

Type I, dimeric isoquinolinequinones, is a system of formula (VIII) most commonly occurring in this class of compounds, see the following table I.

Table I

Structure of Type I Saframycin Antibiotics.

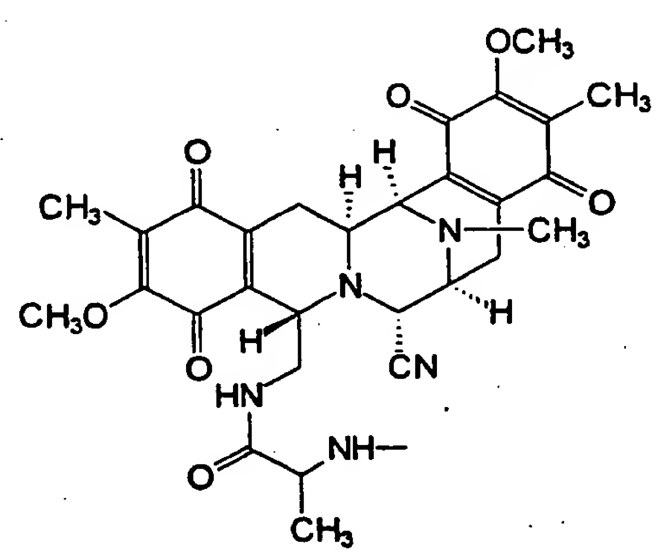


	Substituents	

Compound	R^{14a}	R^{14b}	R^{21}	R^{25a}	R^{25b}	R^{25c}
saframycin A	H	H	CN	O	O	CH_3
saframycin B	H	H	H	O	O	CH_3
saframycin C	H	OCH_3	H	O	O	CH_3
saframycin G	H	OH	CN	O	O	CH_3
saframycin H	H	H	CN	OH	CH_2COCH_3	CH_3
saframycin S	H	H	OH	O	O	CH_3
saframycin Y_3	H	H	CN	NH_2	H	CH_3
saframycin Yd_1	H	H	CN	NH_2	H	C_2H_5
saframycin Ad_1	H	H	CN	O	O	C_2H_5
saframycin Yd_2	H	H	CN	NH_2	H	H
saframycin Y_{2b}	H	Q^b	CN	NH_2	H	CH_3
saframycin $Y_{2b\text{-}d}$	H	Q^b	CN	NH_2	H	C_2H_5
saframycin AH_2	H	H	CN	H^a	OH^a	CH_3
saframycin AH_2Ac	H	H	CN	H	OAc	CH_3
saframycin AH_1	H	H	CN	OH^a	H^a	CH_3
saframycin AH_1Ac	H	H	CN	OAc	H	CH_3
saframycin AR_3	H	H	H	H	OH	CH_3

[a] assignments are interchangeable.

[b] where the group Q is of formula (IX):



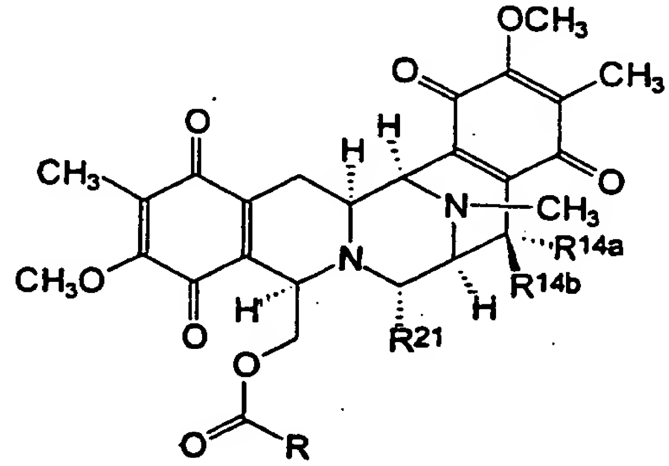
Type I aromatic rings are seen in saframycins A, B and C; G and H; and S isolated from *Streptomyces lavendulae* as minor components.

A cyano derivative of saframycin A, called cyanoquinonamine, is known from Japanese Kokai JP-A2 59/225189 and 60/084288. Saframycins Y_3, Yd_1, Ad_1, and Yd_2 were produced by *S. lavendulae* by directed biosynthesis, with appropriate supplementation of the culture medium. Saframycins Y_{2b} and Y_{2b-d} dimers formed by linking the nitrogen on the C-25 of one unit to the C-14 of the other, have also been produced in supplemented culture media of *S. lavendulae*. Saframycins AR_1 (=AH_2,), a microbial reduction product of saframycin A at C-25 produced by *Rhodococcus amidophilus*, is also prepared by nonstereoselective chemical reduction of saframycin A by sodium borohydride as a 1:1 mixture of epimers followed by chromatographic separation [the other isomer AH_1 is less polar]. The further reduction product saframycin AR_3, 21-decyano-25-dihydro-saframycin A, (= 25-dihydrosaframycin B) was produced by the same microbial conversion. Another type of microbial conversion of saframycin A using a *Nocardia* species produced saframycin B and further reduction by a *Mycobacterium* species produced saframycin AH^1Ac. The 25-*O*-acetates of saframycin AH_2 and AH_1 have also been prepared chemically for biological studies.

Type I compounds of formula (X) have also been isolated from marines sponges, see Table II.

Table II

Structures of Type I Compounds from Marine Sponges.



	Substituents			
	R^{14a}	R^{14b}	R^{21}	R
renieramycin A	OH	H	H	$-C(CH_3)=CH-CH_3$
renieramycin B	OC_2H_5	H	H	$-C(CH_3)=CH-CH_3$
renieramycin C	OH	O	O	$-C(CH_3)=CH-CH_3$
renieramycin D	OC_2H_5	O	O	$-C(CH_3)=CH-CH_3$
renieramycin E	H	H	OH	$-C(CH_3)=CH-CH_3$
renieramycin F	OCH_3	H	OH	$-C(CH_3)=CH-CH_3$
xestomycin	OCH_3	H	H	$-CH_3$

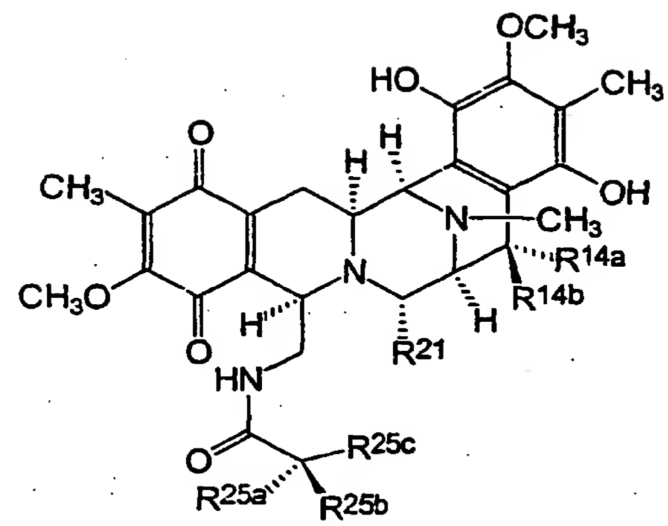
Renieramycins A-D were isolated from the antimicrobial extract of a sponge, a *Reniera* species collected in Mexico, along with the biogenetically related monomeric isoquinolines renierone and related compounds. The structure of renieramycin A was initially assigned with inverted stereochemistry at C-3, C-11, and C-13. However, careful examination of the ^1H NMR data for new, related compounds renieramycins E and F, isolated from the same sponge collected in Palau, revealed that the ring junction of renieramycins was identical to that of saframycins. This result led to the conclusion that the formerly assigned stereochemistry of renieramycins A to D must be the same as that of saframycins.

Xestomycin was found in a sponge, a *Xestospongia* species collected from Sri Lancan waters.

Type II compounds of formula (XI) with a reduced hydroquinone ring include saframycins D and F, isolated from *S. lavendulae*, and saframycins Mx-1 and Mx-2, isolated from *Myxococcus xanthus*. See table III.

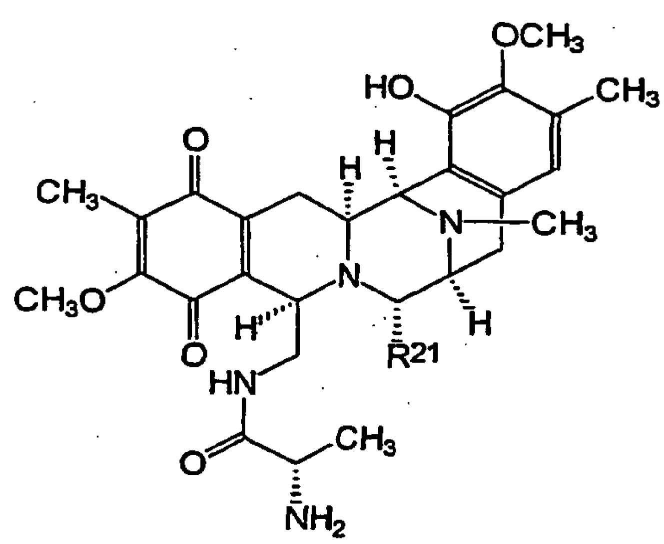
Table III

Type II Compounds



Compound	Substituents R^{14a}	R^{14b}	R^{21}	R^{25a}	R^{25b}	R^{25c}
saframycin D	O	O	H	O	O	CH_3
saframycin F	O	O	CN	O	O	CH_3
saframycin Mx-1	H	OCH_3	OH	H	CH_3	NH_2
saframycin Mx-2	H	OCH_3	H	H	CH_3	NH_2

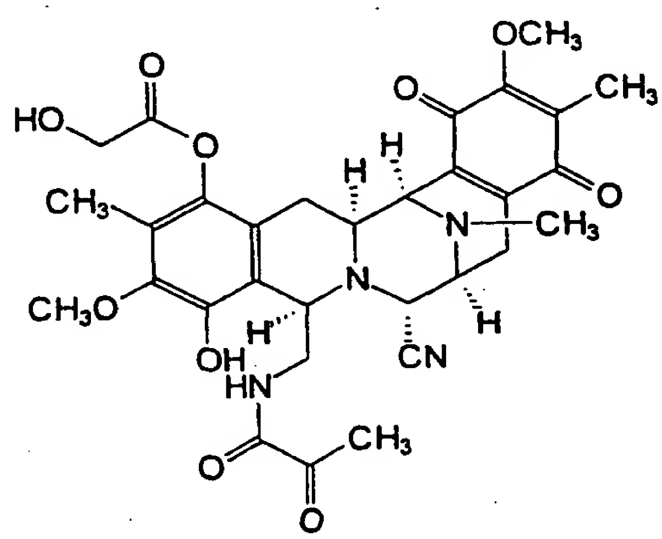
The type III skeleton is found in the antibiotics safracins A and B, isolated from cultured *Pseudomonas fluorescens*. These antibiotics of formula (XII) consist of a tetrahydroisoquinoline-quinone subunit and a tetrahydroisoquninolinephenol subunit.



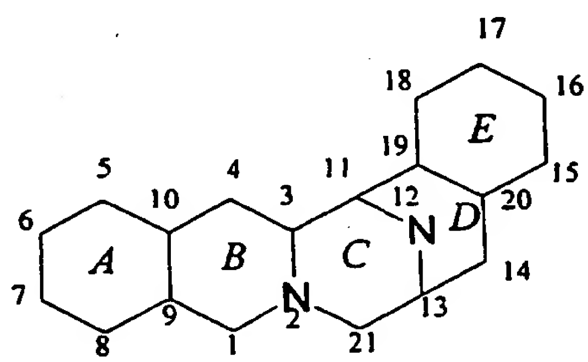
where R^{21} is -H in safracin A and is -OH in safracin B.

Saframycin R, the only compound classified as the Type IV skeleton, was also isolated from *S. lavendulae*. This compound of

formula (XIII), consisting of a hydroquinone ring with a glycolic ester sidechain on one of the phenolic oxygens, is conceivably a pro-drug of saframycin A because of its moderate toxicity.



All these known compounds have a fused system of five rings (*A*) to (*E*) as shown in the following structure of formula (XIV):



The rings *A* and *E* are phenolic in the ecteinascidins and some other compounds, while in other compounds, notably the saframycins, the rings *A* and *E* are quinolic. In the known compounds, the rings *B* and *D* are tetrahydro, while ring *C* is perhydro.

Object of the Invention

The need remains for alternative synthetic routes to the ecteinascidin compounds and related compounds. Such synthetic

routes may provide more economic paths to the known antitumour agents, as well as permitting preparation of new active compounds.

SUMMARY OF THE INVENTION

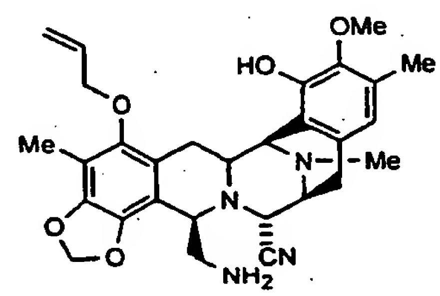
This invention relates to synthetic processes for the formation of intermediates, derivatives and related structures of ecteinascidin or other tetrahydroisoquinolinephenol compounds.

In one aspect, the present invention provides a process for preparing an ecteinascidin product with a spiroamine-1,4-bridge. The process involving forming a 1,4 bridge using a 1-labile, 10-hydroxy, 18-protected hydroxy, di-6,8-en-5-one fused ring compound, where the fused ring is the formula (XIV). In the present invention, the C-18 protection is removed before spiroamine introduction.

Suitable starting materials for the new synthetic processes include compounds related to the natural bis(tetrahydroisoquinoline) alkaloids. Such starting materials may be prepared either from the different classes of saframycin and safracin antibiotics available from different culture broths as detailed in WO 0069862 or by other synthetic or biochemical processes. In this respect, WO 0069862 is incorporated herein in full by reference. The present PCT application claims priority from application PCT/GB 00/01852 which was published as WO 0069862. We incorporate that text by reference to the extent that there is disclosure therein which is not in the present specification.

PREFERRED EMBODIMENTS OF THE INVENTION

In one particular aspect, the present invention is directed at the use of the compound Intermediate 21 in a number of new synthetic processes for the preparation of ecteinascidin 743 and related compounds,



21

The Intermediate 21 has a 5-allyloxy group, where the allyl group serves to protect the 5-hydroxy group. It will be understood that other protecting groups can easily be employed, and that the present invention extends generally to the use of other such 5-protected hydroxy compounds.

FORMATION OF ECTEINASCIDIN 743 AND RELATED COMPOUNDS

In general, the conversion of Intermediate 21, or a related compound, to an ecteinascidin product involves the following key transformations:

(a) Conversion of the NH_2 to OH by reaction, for example with sodium nitrite in acetic acid.

(b) E-ring phenol protection.

(c) Esterification by protecting the primary 1-hydroxy function with a protected cysteine sidechain.

(d) Deprotection of allyl group and oxidation.

(e) Creation of the bridged ring by cyclization reaction.

(f) Deprotections of E-ring phenol and the cysteine moiety

(g) Quinoline Introduction by Trans-amination and Petter Spengler reactions.

The high functionality of the intermediate compounds necessitates the use of protecting groups for the E-ring phenol and for the cysteine sidechain in order to prevent unwanted side reactions.

As such, a number of alternative intermediates can be generated dependent on the particular selection of protecting groups.

Different possible sequences are possible for combining these transformations dependent primarily on the protecting groups selected for the phenol ring and for the amine of the cysteine sidechain.

The total number of synthetic transformations is also a function of the protecting groups selected.

By way of illustration, the use of different combinations of protecting groups is described below for six typical routes for the preparation of ET-743 from Intermediate 21, also referred to herein as SF21.

Route	*Phenol Protection*	*Cysteine Protection*	*Number of steps*
1	MOM	Boc	12
2	MEM	Boc	10

3	MEM	Cbz	11
4	MOM	Alloc	13
5	MEM	Alloc	13
6	MOM	Cbz	15

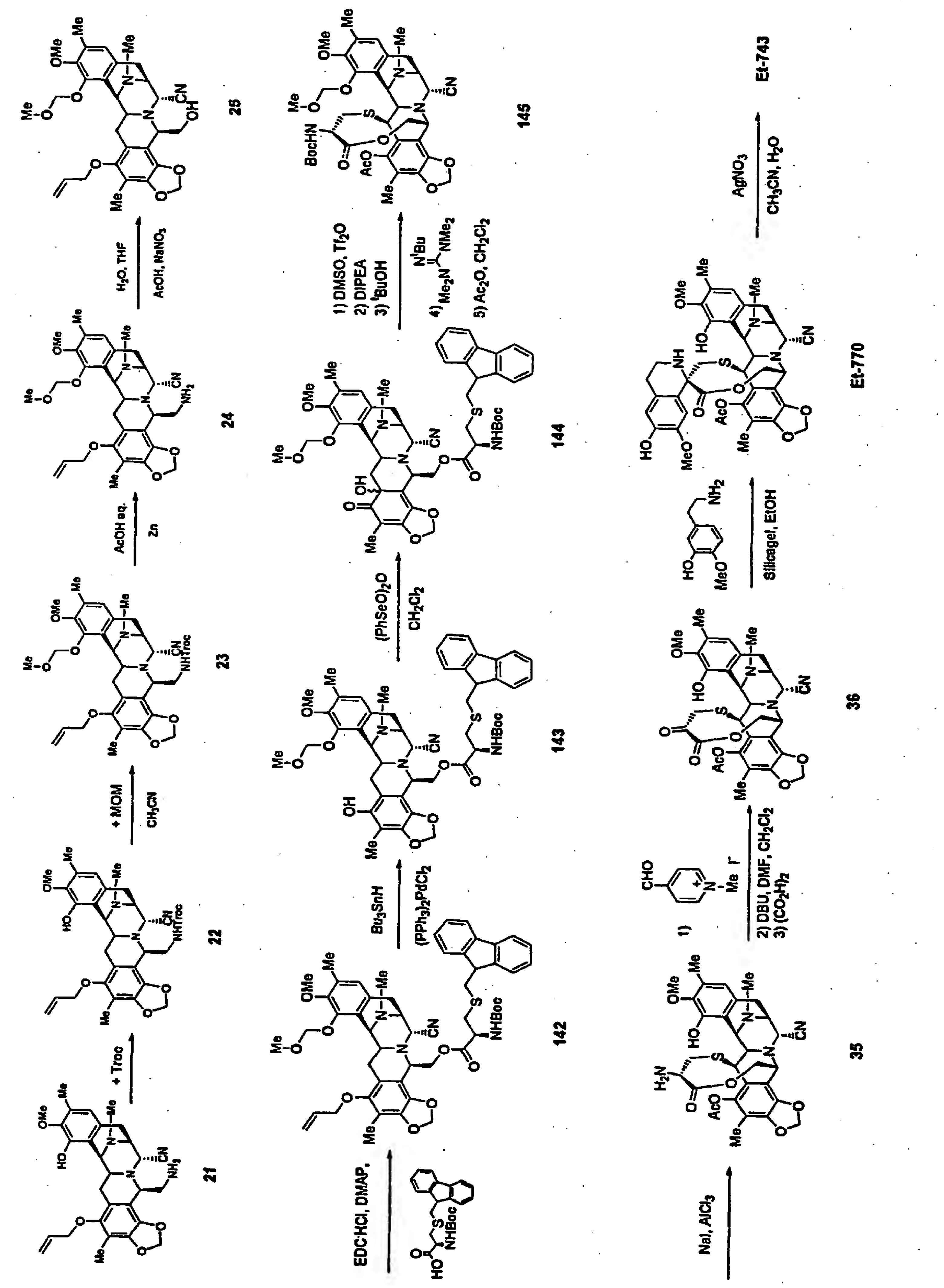
As the skilled artisan will readily appreciate, the reaction schemes described herein may be modified and/or combined in various ways, and the alternative sequences of steps and the compounds generated therefrom are part of this invention.

Additionally, the use of other protecting group strategies not detailed is also part of this invention.

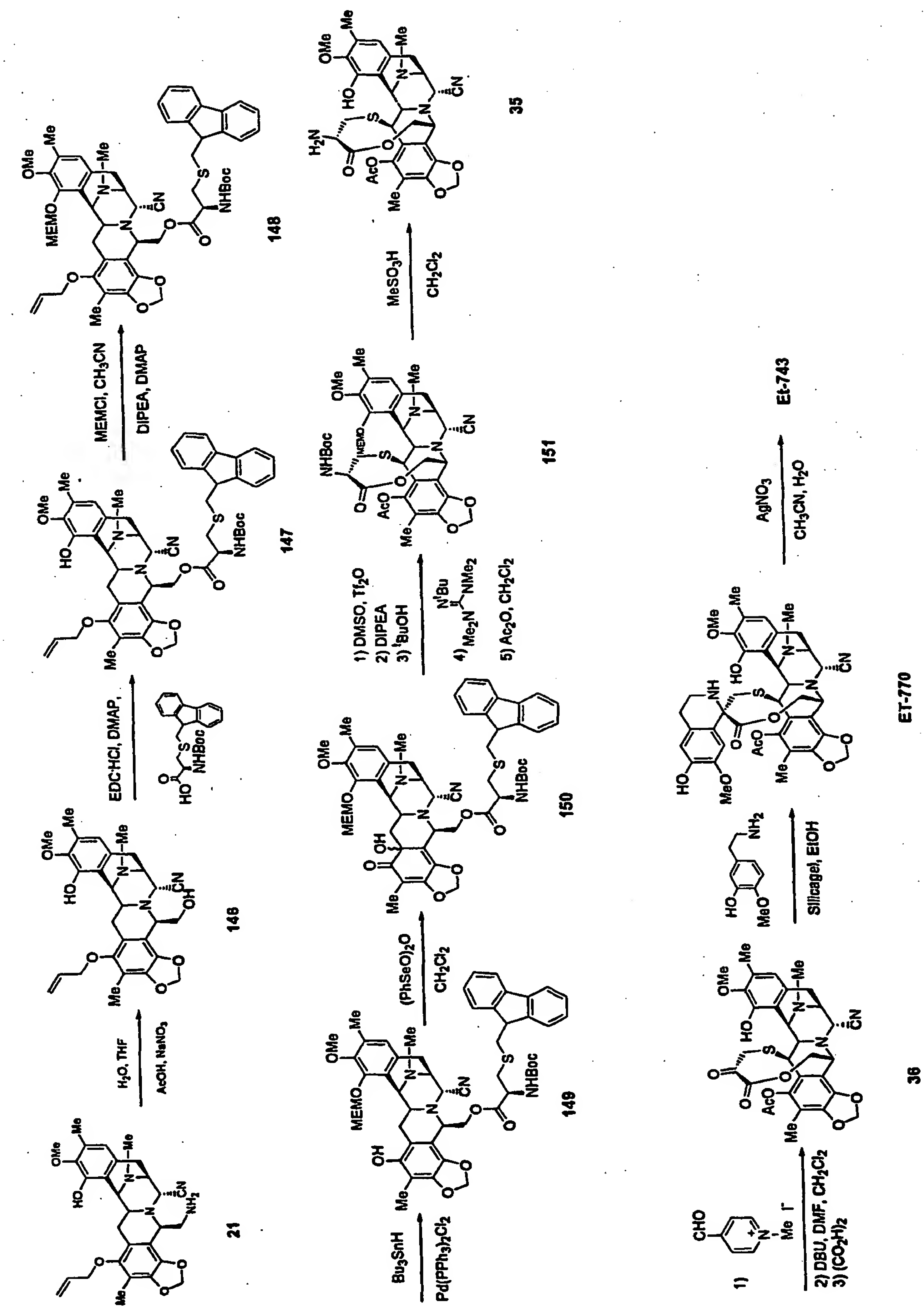
PROCESS DETAILS OF SIX TYPICAL SYNTHETIC ROUTES

Full reaction schemes for each route are in the following Schemes 1 to 6.

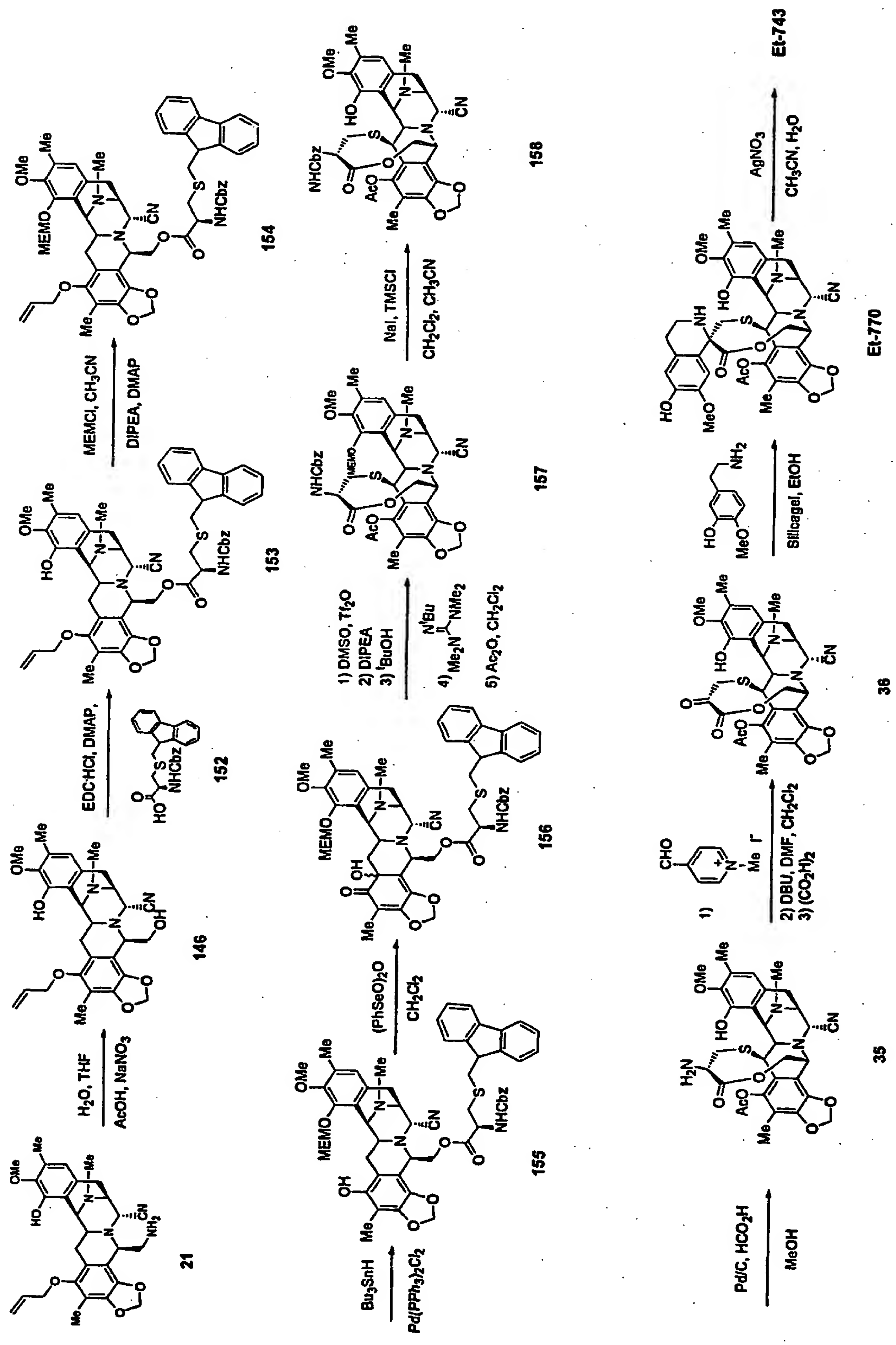
Scheme 1 – ET-743: Hemisynthetic Alternative Route 1



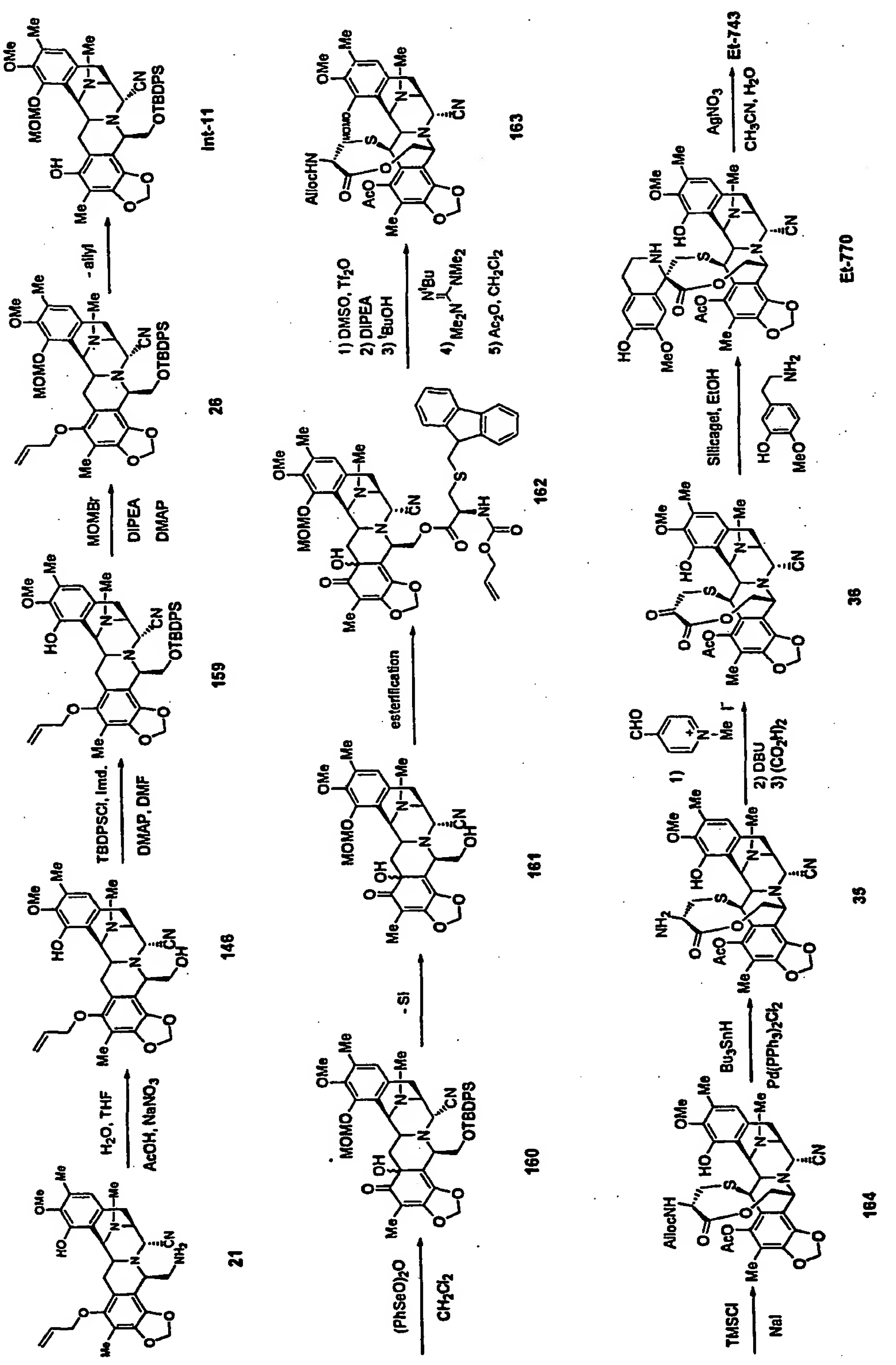
Scheme 2 – ET-743: Hemisynthetic Alternative Route 2



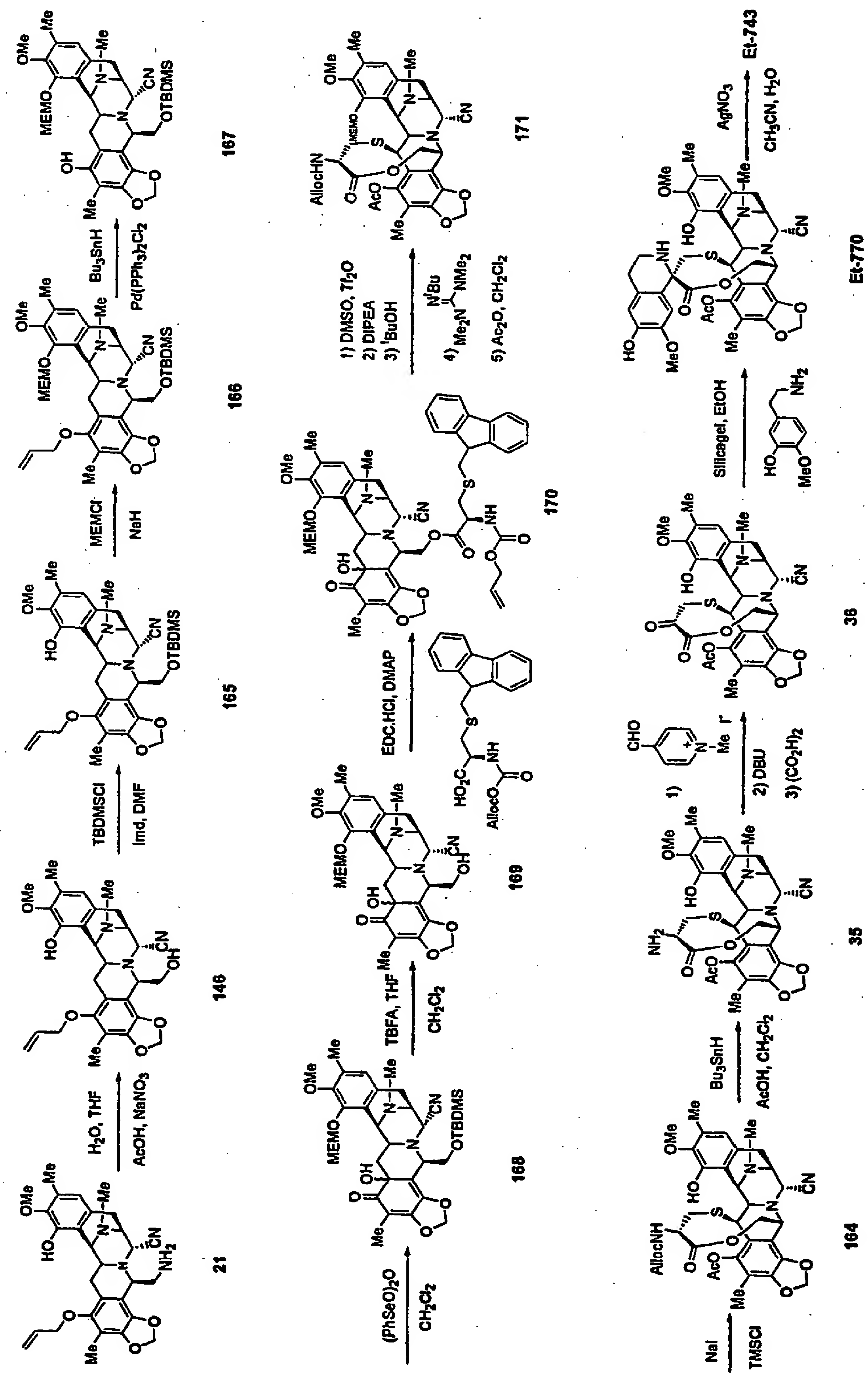
Scheme 3 – ET-743: Hemisynthetic Alternative Route 3



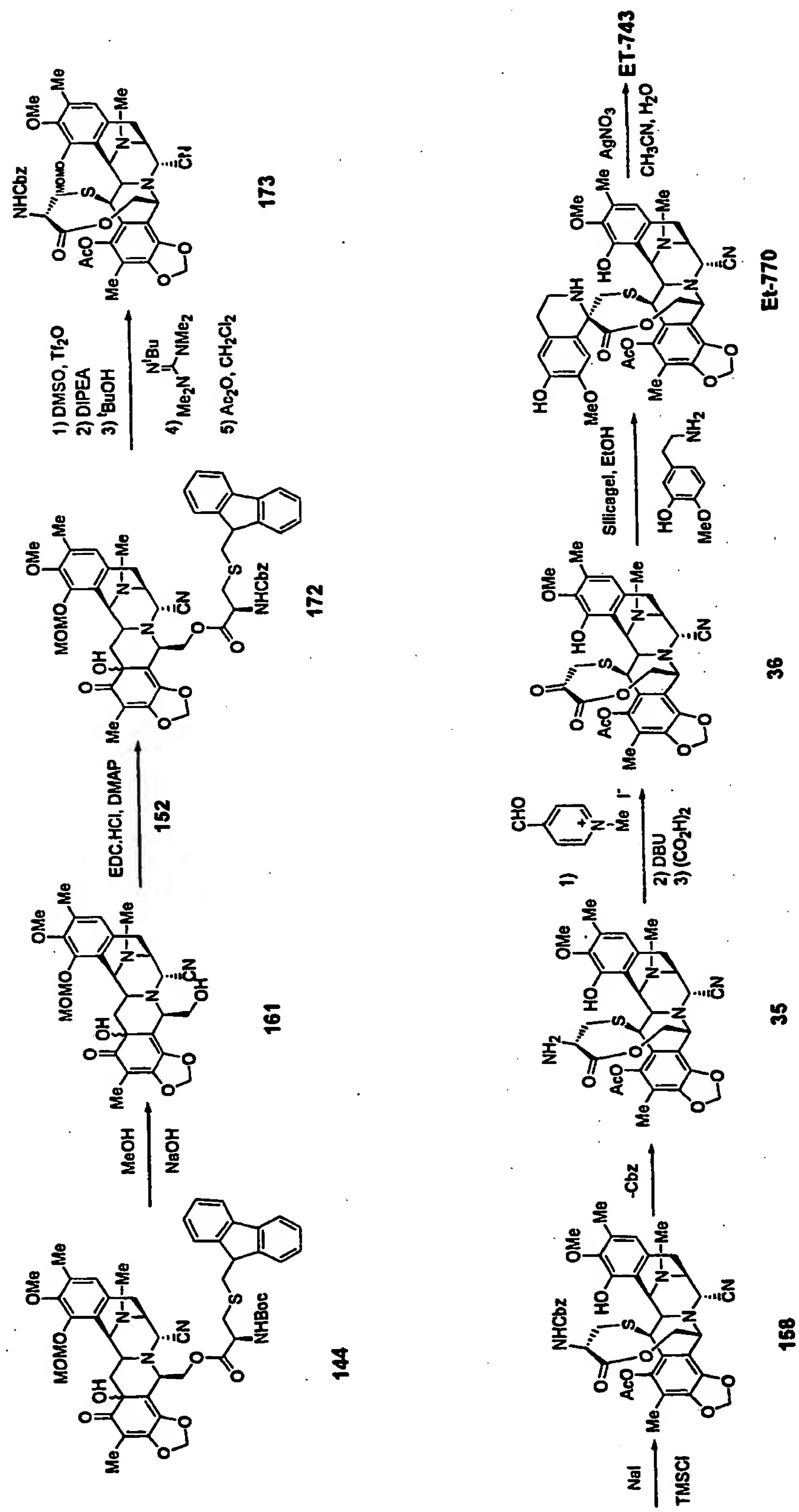
Scheme 4- ET-743: Hemisynthetic Alternative Route 4



Scheme 5- ET-743: Hemisynthetic Alternative Route 5



Scheme 6 – ET-743 Hemisynthetic Alternative Route 6



In route 1, protection of the E-ring phenol is achieved in three steps involving protection/deprotection of the amine of SF21 with Troc.

For routes 1 and 2, protection of the cysteine sidechain with Boc allows the phenol and cysteine groups to be deprotected in a single step rather than as two separate steps. For the rest of the routes, an additional deprotection step is required.

For route 2, Intermediate 25 is avoided through the use of the direct esterification methodology and the subsequent protection of the phenol with the MEM group.

In routes 2 and 3 protection of the E-ring phenol is delayed until after the diazotisation and esterification steps thereby allowing the phenol to be protected in a single step rather than by the three step sequence of route 1.

For routes 1, 2 and 3, direct esterification of the primary alcohol with the cysteine derivative eliminates the unproductive protection/deprotection steps of the primary alcohol with a silyl group (routes 4 and 5) thereby shortening the sequence by two steps.

Route 6 only contemplates herein the last steps from intermediate **161**, which can be easily obtained from intermediate **21**.

In routes 4 and 5 the primary alcohol produced by the initial diazotisation step is protected with silicon to allow selective protection of the E-ring phenol and avoiding intermediate 25. Following

modification of the A-ring (deprotection/oxidation), the silicon group is removed and the primary alcohol esterified with the cysteine derivative.

These changes are a direct consequence of the problems that were found in the scale up of the route given in WO 0069862. As a result of these changes the overall route 2 is three steps shorter and potentially therefore more suitable and/or cheaper for routine manufacture.

PROCESS OVERVIEW

Thus, in view of the routes 1 to 6, the present invention extends to a process for preparing an ecteinascidin product with a spiroamine-1,4-bridge, the process involving forming a 1,4 bridge using a 1-labile, 10-hydroxy, 18-protected hydroxy, di-6,8-en-5-one fused ring compound, wherein C-18 protection is removed before spiroamine introduction.

In one version of the process, the ecteinascidin product has a 21-hydroxy group, and the process includes converting a 21-cyano group to the 21-hydroxy group.

Typically the spiroamine is a spiroquinoline, especially the spiroquinoline of ecteinascidin 743.

In a preferred process the 18-protected group of the 1-labile, 10-hydroxy, 18-protected hydroxy, di-6,8-en-5-one fused ring compound is protected with: MOM, methoxymethyl; or MEM, methoxyethoxymethyl group.

Suitably the 1-labile group is an N-protected cysteinyloxymethylene group of the formula

$-CH_2-O-CO-CNHProt^1-CH_2-S-H$.

In this formula Prot[1] is typically: Boc, t-butyloxycarbonyl; Troc, 2,2,2-trichloroethyloxycarbonyl; Cbz, benzyloxycarbonyl; or Alloc, allyloxycarbonyl.

With some embodiments of the process, Prot[1] is removed in the same step as C-18 protection.

The 1-labile group can be generated from a 1-substituent of the formula:

$-CH_2-O-CO-CNHProt^1-CH_2-S-Prot^2$.

In this formula, Prot[2] is typically Fm, 9-fluorenylmethyl.

A 1-substituent of the formula:

$-CH_2-O-CO-CNHProt^1-CH_2-S-Prot^2$

can be formed by esterification of a $-CH_2-O-H$ substituent.

The esterification can be carried out before or after formation of the 10-hydroxy, di-6,8-en-5-one structure.

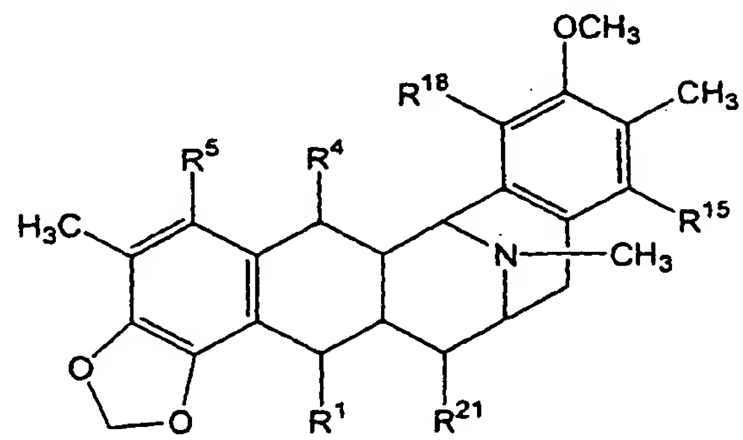
In one version, the claimed process starts from a 1-aminomethylene, 5-protected hydroxy, 7,8-dioxymethylene, 18-hydroxy, 21-cyano fused ring compound

The 1-aminomethylene group can be temporarily protected to allow protection at the 18-hydroxy group, and the temporary protection removed.

Alternatively, the C-18 hydroxy group can be protected after formation of a 1-ester function.

In another variation, the 1-aminomethylene group is converted to a 1-hydroxymethylene group and the 1-hydroxymethylne group is temporarily protected, to allow protection at the 18-hydroxy group, and the temporary protection is removed.

The fused ring structure is suitably of the formula:



,

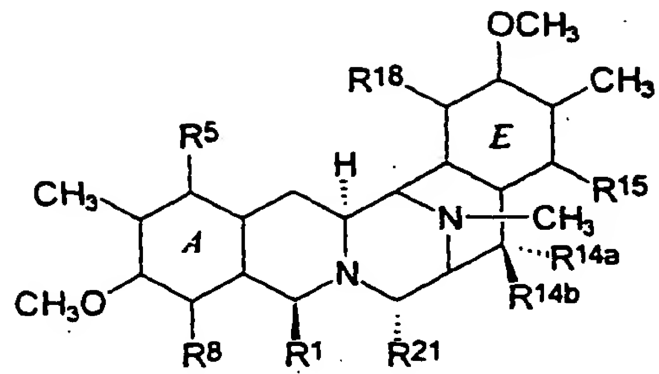
especially where R^{15} is H. One or more or all of the remaining subsitituents can be as in ecteinascidin 743.

HEMISYTHESIS

The invention permits the use of a known compound, safracin B, also referred to as quinonamine, in hemisynthetic synthesis.

More generally, the invention relates to a hemisynthetic process for the formation of intermediates, derivatives and related structures of ecteinascidin or other tetrahydroisoquinolinephenol compounds starting from natural bis(tetrahydroisoquinoline) alkaloids. Suitable starting materials for the hemi-synthetic process include the classes of saframycin and safracin antibiotics available from different culture broths, and also the classes of reineramicin and xestomycin compounds available from marine sponges.

A general formula (XV) for the starting compounds is as follows:



where:

R^1 is an amidomethylene group such as $-CH_2-NH-CO-CR^{25a}R^{25b}R^{25c}$ where R^{25a} and R^{25b} form a keto group or one is $-OH$, $-NH_2$ or $-OCOCH_3$ and the other is $-CH_2COCH_3$, $-H$, $-OH$ or $-OCOCH_3$, provided that when R^{25a} is $-OH$ or $-NH_2$ then R^{25b} is not $-OH$, and R^{25c} is $-H$, $-CH_3$ or $-CH_2CH_3$, or R^1 is an acyloxymethylene group such as $-CH_2-O-CO-R$, where R is $-C(CH_3)=CH-CH_3$ or $-CH_3$;

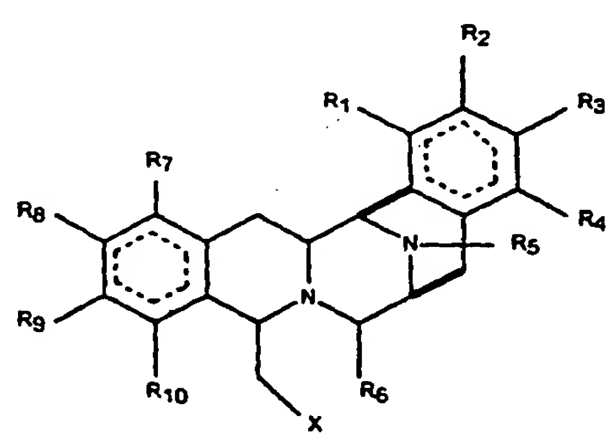
R^5 and R^8 are independently chosen from -H, -OH or $-OCOCH_2OH$, or R^5 and R^8 are both keto and the ring *A* is a p-benzoquinone ring;

R^{14a} and R^{14b} are both -H or one is -H and the other is -OH, $-OCH_3$ or $-OCH_2CH_3$, or R^{14a} and R^{14b} together form a keto group;

R^{15} and R^{18} are independently chosen from -H or -OH, or R^5 and R^8 are both keto and the ring *A* is a p-benzoquinone ring; and

R^{21} is -OH or -CN.

A more general formula for these class of compounds is provided below:

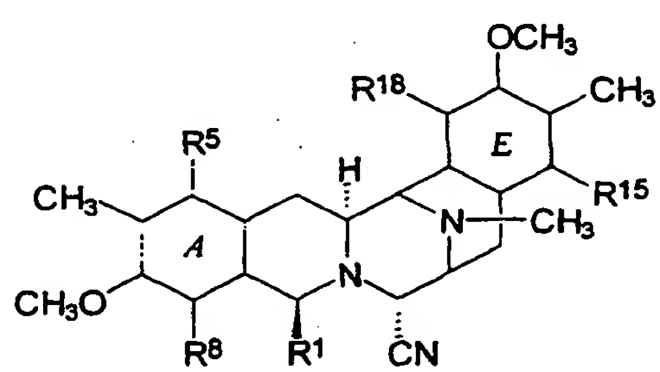


wherein the substituent groups defined by R_1, R_2, R_3, R_4, R_5, R_6, R_7, R_8, R_9, R_{10} are each independently selected from the group consisting of H, OH, OCH_3, CN, =O, CH_3;

wherein X are the different amide or ester functionalities contained in the mentioned natural products;
wherein each dotted circle represents one, two or three optional double bonds.

Thus, according to the present invention, we now provide hemisynthetic routes for the production of intermediates including Intermediates 11 or 21 and thus for the production of the ecteinascidin compounds as well as phthalascidin and additional compounds. The hemisynthetic routes of the invention each comprise a number of transformation steps to arrive at the desired product. Each step in itself is a process in accordance with this invention. The invention is not limited to the routes that are exemplified, and alternative routes may be provided by, for example, changing the order of the transformation steps, as appropriate or by a change to the protecting groups used.

In particular, this invention involves the provision of a 21-cyano starting material of general formula (XVI):



where R^1, R^5, R^8, R^{14a}, R^{14b}, R^{15} and R^{18} are-as-defined.

Other compounds of formula (XVI) with different substituents at the 21-position may also represent possible starting materials. In general, any derivative capable of production by nucleophilic displacement of the 21-hydroxy group of compounds of formula (XV) wherein R^{21} is a hydroxy group cis a candidate. Examples of suitable 21-substituents include but are not limited to:

a mercapto group;

an alkylthio group (the alkyl group having from 1 to 6 carbon atoms);

an arylthio group (the aryl group having from 6 to 10 carbon atoms and being unsubstituted or substituted by from 1 to 5 substituents selected from, for example, alkyl group having from 1 to 6 carbon atoms, alkoxy groups having from 1 to 6 carbon atoms, halogen atoms, mercapto groups and nitro groups);

an amino group;

a mono-or dialkylamino (the or each alkyl group having from 1 to 6 carbon atoms);

a mono-or diarylamino group (the or each aryl group being as defined above in relation to arylthio groups);

an α-carbonylalkyl group of formula $-C(R^a)(R^b)-C(=O)R^c$, where R^a and R^b are selected from hydrogen atoms, alkyl groups having from 1 to 20 carbon atoms, aryl groups (as defined above in relation to arylthio groups) and aralkyl groups (in which an alkyl group having from 1 to 4 carbon atoms is substituted by an aryl group a defined above in relation to arylthio groups), with the proviso that one of R^a and R^b is a hydrogen atom;

R^c is selected from a hydrogen atom, an alkyl group having from 1 to 20 carbon atoms, aryl groups (as defined above in relation to arylthio groups), an aralkyl group (in which an alkyl group having from 1 to 4 carbon atoms is substituted by an aryl group a defined above in relation to arylthio groups), an alkoxy group having from 1 to 6 carbon atoms, an amino group or a mono- or dialkylamino group as defined above.

Thus, in a more general aspect, the present invention relates to processes where the first step is to form a 21-deriviative using a nucleophilic reagent. We refer to such compounds as 21-Nuc compounds.

The presence of the 21-cyano group is required for some of the end-products, notably ecteinascidin 770 and phthalascidin, while for other end-products it acts as a protecting group which can readily be converted to another substituent, such as the 21-hydroxy group of ecteinascidin 743 or of 21-hydroxyphthalascidin. The adoption of the 21-cyano compound as the starting material effectively stabilises the molecule during the ensuing synthetic steps, until it is optionally removed. Other 21-Nuc compounds can offer this and other advantages.

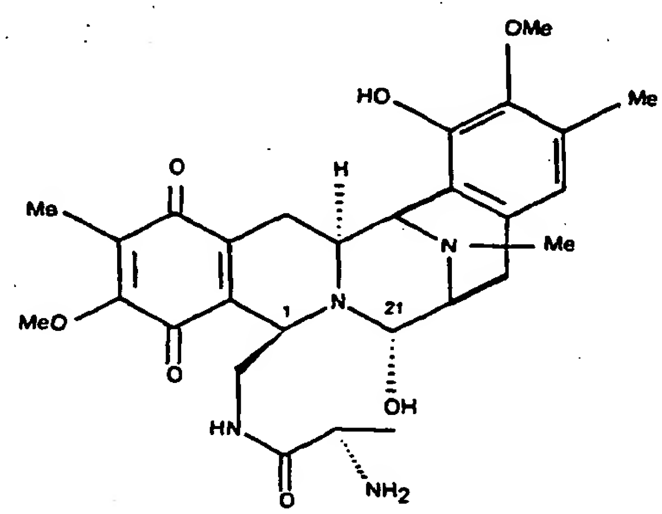
In one important aspect, the present invention consists in the use of a 21-cyano compound of the general formula (XVI) in the preparation of a bis- or tris-(tetrahydroisoquinolinephenol) compounds. Products which may be prepared include intermediates such as Intermediate 11 or 21, and the ecteinascidins, as well as new and known compounds of related structure.

Preferred starting materials include those compounds of formula (XV) or (XVI) where R^{14a} and R^{14b} are both hydrogen. Preferred starting materials also include compounds of formula (XV) or (XVI) where R^{15} is hydrogen. Furthermore, the preferred starting materials include compounds of formula (XV) or (XVI) where ring *E* is a phenolic ring. Preferred starting materials further include compounds of formula (XV) or (XVI) where at least one, better at least two or three of R^5, R^8, R^{15} and R^{18} is not hydrogen.

Examples of suitable starting materials for this invention include saframycin A, saframycin B, saframycin C, saframycin G, saframycin H, saframycin S, saframycin Y_3, saframycin Yd_1, saframycin Ad_1, saframycin Yd_2, saframycin AH_2, saframycin AH_2Ac, saframycin AH_1, saframycin AH_1Ac, saframycin AR_3, renieramycin A, renieramycin B, renieramycin C, renieramycin D, renieramycin E, renieramycin F,

xestomycin, saframycin D, saframycin F, saframycin Mx-1, saframycin Mx-2, safracin A, safracin B and saframycin R. Preferred starting materials have a cyano group in position 21, for the group R^{21}.

In a particularly preferred aspect, the invention involves a hemisynthetic process wherein the transformation steps are applied to safracin B:



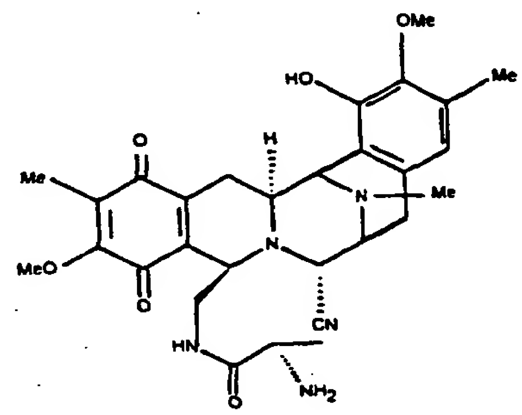
SAFRACIN B

Safracin B presents a ring system closely related to the ecteinascidins. This compound has the same pentacycle structure and the same substitution pattern in the right-hand aromatic ring, ring *E*. Also, safracin B presents very close similarities to some of the synthetic intermediates in the total synthesis of ET-743, particularly to the intermediates 11 or 21. Such intermediate can be transformed into Et-743 using a well established method. Synthetic conversion of safracin B into intermediates 11 or 21 will therefore provide an hemi-synthetic method to obtain ET-743.

Thus, we provide Intermediates 11 or 21 made from this compound safracin B, and compounds derived from Intermediate 11 or 21, particularly ecteinascidin compounds. We further provide phthalascidin made from safracin B. The invention also relates to use of safracin B in the production of Intermediates 11 or 21, ecteinascidin compounds and the other intermediates of the invention. The

invention also relates to compounds described herein derived from the other suggested starting materials, and use of those compounds in the production of such compounds.

The more preferred starting materials of this invention have a 21-cyano group. The currently most preferred compound of the present invention is the compound of Formula **2**. This compound is obtained directly from safracin B and is considered a key intermediate in the hemisynthetic process.



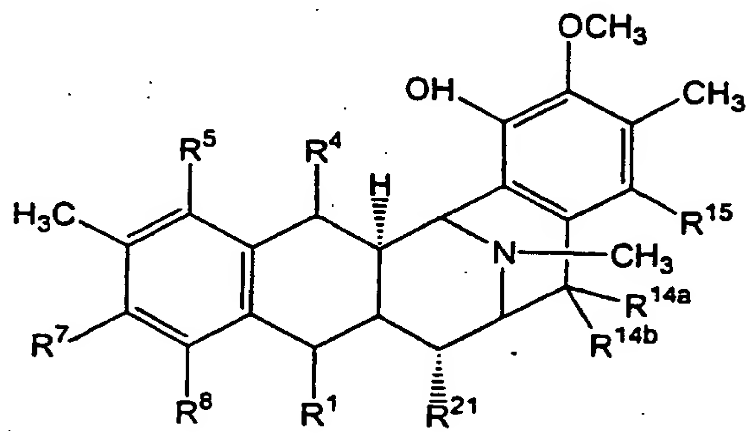
compound 2

In a related aspect, we provide cyanosafracin B by fermentation of a safracin B-producing strain of *Pseudomonas fluorescens*, and working up the cultured broth using cyanide ion. The preferred strain of *Pseudomonas fluorescens* is strain A2-2, FERM BP-14, which is employed in the procedure of EP 055,299. A suitable source of cyanide ion is potassium cyanide. In a typical work-up, the broth is filtered and excess cyanide ion is added. After an appropriate interval of agitation, such as 1 hour, the pH is rendered alkaline, say pH 9.5, and an organic extraction gives a crude extract which can be further purified to give the cyanosafracin B.

For the avoidance of doubt, the stereochemistries indicated in this patent specification are based on our understanding of the correct stereochemistry of the natural products. To the extent that an error is discovered in the assigned stereochemistry, then the appropriate

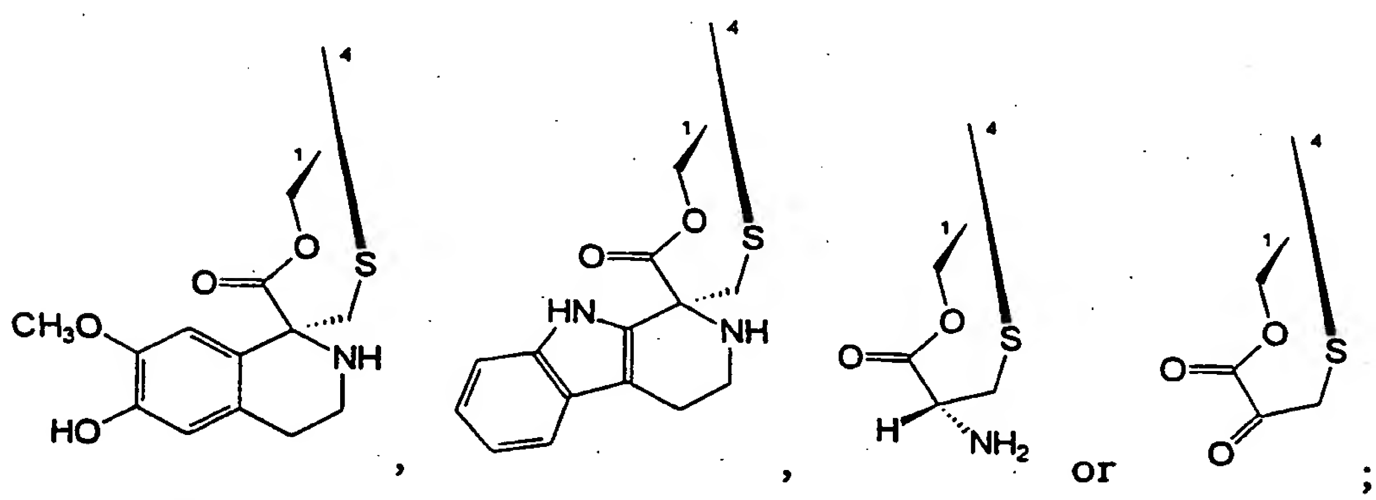
correction needs to be made in the formulae given throughout in this patent specification. Furthermore, to the extent that the syntheses are capable of modification, this invention extends to stereoisomers.

The products of this invention are typically of the formula (XVIIb):



where

R^1 and R^4 together form a group of formula (IV), (V) (VI) or (VII):



R^5 is -H or -OH;

R^7 and R^8 together form a group $-O-CH_2-O-$;

R^{14a} and R^{14b} are both -H or one is -H and the other is -OH, $-OCH_3$ or $-OCH_2CH_3$, or R^{14a} and R^{14b} together form a keto group; and

R^{15} is -H or -OH;

R^{21} is -H, -OH or -CN;

and derivatives including acyl derivatives thereof especially where R^5 is acetyloxy or other acyloxy group of up to 4 carbon atoms.

In the formula (XVIIb), R^1 typically with R^4 forms a group (IV) or (V). The group R^{18} is usually protected. Usually R^{21} is cyano.

Preferably R^{14a} and R^{14b} are hydrogen. Preferably R^{15} is hydrogen. The O-acyl derivatives are suitably aliphatic O-acyl derivatives, especially acyl derivatives of 1 to 4 carbon atoms, and typically an O-acetyl group, notably at the 5-position.

Suitable protecting groups for phenols and hydroxy groups include ethers and esters, such as alkyl, alkoxyalkyl, aryloxyalkyl, alkoxyalkoxyalkyl, alkylsilylalkoxyalkyl, alkylthioalkyl, arylthioalkyl, azidoalkyl, cyanoalkyl, chloroalkyl, heterocyclic, arylacyl, haloarylacyl, cycloalkylalkyl, alkenyl, cycloalkyl, alyklarylalkyl, alkoxyarylalkyl, nitroarylalkyl, haloarylalkyl, alkylaminocarbonylarylalkyl, alkylsulfinylarylalky, alkylsilyl and other ethers, and arylacyl, aryl alkyl carbonate, aliphatic carbonate, alkylsulfinylarlyalkyl carbonate, alkyl carbonate, aryl haloalkyl carbonate, aryl alkenyl carbonate, aryl carbamate, alkyl phosphinyl, alkylphosphinothioyl, aryl phosphinothioyl, aryl alkyl sulphonate and other esters. Such groups may optionally be substituted with the previously mentioned groups in R^1.

Suitable protecting groups for amines include carbamates, amides, and other protecting groups, such as alkyl, arylalkyl, sulpho- or halo- arylalkyl, haloalkyl, alkylsilylalkyl, arylalkyl, cycloalkylalkyl, alkylarylalkyl, heterocyclylalkyl, nitroarylalkyl, acylaminoalkyl, nitroaryldithioarylalkyl, dicycloalkylcarboxamidoalkyl, cycloalkyl, alkenyl, arylalkenyl, nitroarylalkenyl, heterocyclylalkenyl, heterocyclyl, hydroxyheterocyclyl, alkyldithio, alkoxy- or halo- or alkylsulphinyl arylalkyl, hetercyclylacyl, and other carbamates, and alkanoyl, haloalkanoyl, arylalkanoyl, alkenoyl, heterocyclylacyl, aroyl, arylaroyl, haloaroyl, nitroaroyl, and other amides, as well as alkyl, alkenyl, alkylsilylalkoxyalkyl, alkoxyalkyl, cyanoalkyl, heterocyclyl, alkoxyarylalkyl, cycloalkyl, nitroaryl, arylalkyl, alkoxy- or hydroxy-

arylalkyl, and many other groups. Such groups may optionally be substituted with the previously mentioned groups in R^1.

Examples of such protecting groups are given in the following tables.

protection for -OH group

ethers	abbreviation
methyl	
methoxymethyl	MOM
benzyloxymethyl	BOM
methoxyethoxymethyl	MEM
2-(trimethylsilyl)ethoxymethyl	SEM
methylthiomethyl	MTM
phenylthiomethyl	PTM
azidomethyl	
cyanomethyl	
2,2-dichloro-1,1-difluoroethyl	
2-chloroethyl	
2-bromoethyl	
tetrahydropyranyl	THP
1-ethoxyethyl	EE
phenacyl	
4-bromophenacyl	
cyclopropylmethyl	
allyl	
propargyl	
isopropyl	
cyclohexyl	
t-butyl	

benzyl	
2,6-dimethylbenzyl	
4-methoxybenzyl	MPM or PMB
o-nitrobenzyl	
2,6-dichlorobenzyl	
3,4-dichlorobenzyl	
4-(dimethylamino)carbonylbenzyl	
4-methylsuflinylbenzyl	Msib
9-anthrylmethyl	
4-picolyl	
heptafluoro-*p*-tolyl	
tetrafluoro-4-pyridyl	

trimethylsilyl	TMS
t-butyldimethylsilyl	TBDMS
t-butyldiphenylsilyl	TBDPS
triisopropylsilyl	TIPS

esters

aryl formate	
aryl acetate	
aryl levulinate	
aryl pivaloate	ArOPv
aryl benzoate	
aryl 9-fluorocarboxylate	

aryl methyl carbonate	
1-adamantyl carbonate	
t-butyl carbonate	BOC-OAr
4-methylsulfinylbenzyl carbonate	Msz-Oar
2,4-dimethylpent-3-yl carbonate	Doc-Oar

aryl 2,2,2-trichloroethyl carbonate
aryl vinyl carbonate
aryl benzyl carbonate
aryl carbamate

dimethylphosphinyl	Dmp-OAr
dimethylphosphinothioyl	Mpt-OAr
diphenylphosphinothioyl	Dpt-Oar

aryl methanesulfonate
aryl toluenesulfonate
aryl 2-formylbenzenesulfonate

protection for the -NH_2 group

carbamates	abbreviation
methyl	
ethyl	
9-fluorenylmethyl	Fmoc
9-(2-sulfo)fluroenylmethyl	
9-(2,7-dibromo)fluorenylmethyl	
17-tetrabenzo[*a,c,g,i*]fluorenylmethyl	Tbfmoc
2-chloro-3-indenylmethyl	Climoc
benz[*f*]inden-3-ylmethyl	Bimoc
2,7-di-*t*-butyl[9-(10,10-dioxo-10,10,10,10-tetrahydrothioxanthyl)]methyl	DBD-Tmoc
2,2,2-trichloroethyl	Troc
2-trimethylsilylethyl	Teoc
2-phenylethyl	hZ

1-(1-adamantyl)-1-methylethyl	Adpoc
2-chlooethyl	
1,1-dimethyl-2-chloroethyl	
1,1-dimethyl-2-bromoethyl	
1,1-dimethyl-2,2-dibromoethyl	DB-*t*-BOC
1,1-dimethyl-2,2,2-trichloroethyl	TCBOC
1-methyl-1-(4-biphenyl)ethyl	Bpoc
1-(3,5-di-*t*-butylphenyl)-1-1-methylethyl	*t*-Burmeoc
2-(2'-and 4'-pyridyl)ethyl	Pyoc
2,2-bis(4'-nitrophenyl)ethyl	Bnpeoc
n-(2-pivaloylamino)-1,1-dimethylethyl	
2-[(2-nitrophenyl)dithio]-1-phenylethyl	NpSSPeoc
2-(*n,n*-dicyclohexylcarboxamido)ethyl	
t-butyl	BOC
1-adamantyl	1-Adoc
2-adamantyl	2-Adoc
vinyl	Voc
allyl	Aloc or Alloc
1-isopropylallyl	Ipaoc
cinnamyl	Coc
4-nitrocinnamyl	Noc
3-(3'-pyridyl)prop-2-enyl	Paloc
8-quinolyl	
n-hydroxypiperidinyl	
alkyldithio	
benzyl	Cbz or Z
p-methoxybenzyl	Moz
p-nitrobenzyl	PNZ
p-bromobenzyl	
p-chlorobenzyl	
2,4-dichlorobenzyl	
4-methylsulfinylbenzyl	Msz

9-anthrylmethyl
diphenylmethyl
phenothiazinyl-(10)-carbonyl
n'-*p*-toluenesulfonylaminocarbonyl
n'-phenylaminothiocarbonyl

amides

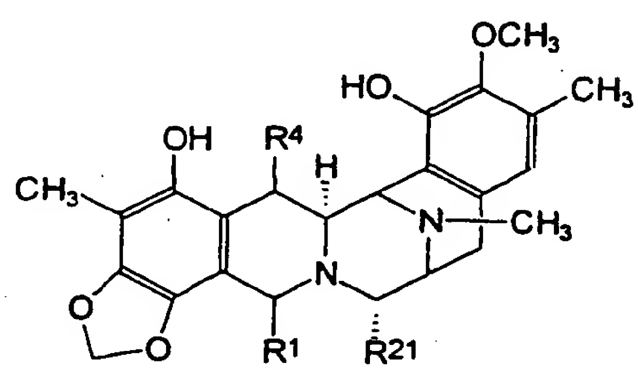
formamide	
acetamide	
chloroacetamide	
trifluoroacetamide	TFA
phenylacetamide	
3-phenylpropanamide	
pent-4-enamide	
picolinamide	
3-pyridylcarboxamide	
benzamide	
p-phenylbenzamide	
n-phthalimide	
n-tetrachlorophthalimide	TCP
4-nitro-*n*-phthalimide	
n-dithiasuccinimide	Dts
n-2,3-diphenylmaleimide	
n-2,5-dimethylpyrrole	
n-2,5-bis(triisopropylsiloxyl)pyrrole	BIPSOP
n-1,1,4,4-tetramethyldisiliazacyclopentante adduct	STABASE
1,1,3,3-tetramethyl-1,3-disilaisoindoline	BSB

special -NH protective groups

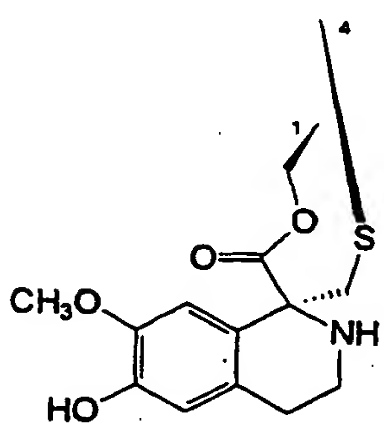
n-methylamine	
n-*t*-butylamine	
n-allylamine	
n-[2-trimethylsilyl)ethoxy]methylamine	SEM
n-3-acetoxypropylamine	
n-cyanomethylamine	
n-(1-isopropyl-4-nitro-2-oxo-3-pyrrolin-3-yl)amine	
n-2,4-dimethoxybenzylamine	Dmb
2-azanorbornenes	
n-2,4-dinitrophenylamine	
n-benzylamine	Bn
n-4-methoxybenzylamine	MPM
n-2,4-dimethoxybenzylamine	DMPM
n-2-hydroxybenzylamine	Hbn
n-(diphenylmethyl)amino	DPM
n-bis(4-methoxyphenyl)methylamine	
n-5-dibenzosuberylamine	DBS
n-triphenylmethylamine	Tr
n-[(4-methoxyphenyl)diphenylmethyl]amino	MMTr
n-9-phenylflurenylamine	Pf
n-ferrocenylmethylamine	Fcm
n-2-picolylamine *n'*-oxide	
n-1,1-dimethylthiomethyleneamine	
n-benzylideneamine	
n-*p*-methoxybenzylideneamine	
n-diphenylmethyleneamine	
n-(5,5-dimethyl-3-oxo-1-cyclohexenyl)amine	
n-nitroamine	
n-nitrosoamine	

diphenylphosphinamide	Dpp
dimethylthiophosphinamide	Mpt
diphenylthiophosphinamide	Ppt
dibenzyl phosphoramidate	
2-nitrobenzenesulfenamide	Nps
n-1-(2,2,2-trifluoro-1,1-diphenyl)ethylsufenamide	TDE
3-nitro-2-pyridinesulfenamide	Npys
p-toluenesulfonamide	Ts
benzenesulfonamide	

Particular ecteinascidin products of this invention include compounds of the formula (XVIII):



where R^1 and R^4 form a group of formula (IV), (V), (VI) or (VII):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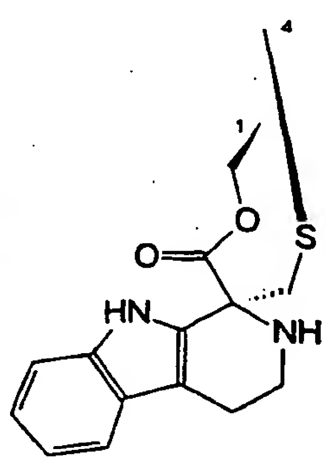
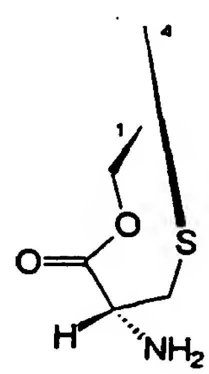
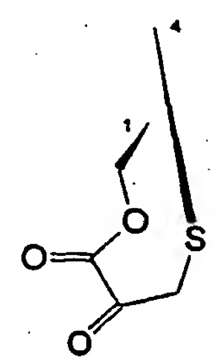

,

or

;

more particularly a group (IV) or (V);

R^{21} is -H, -OH or -CN, more particularly -OH or -CN;

and acyl derivatives thereof, more particularly 5-acyl derivatives including the 5-acetyl derivative.

In general, the conversion of the 21-cyano starting compound to an ecteinascidin product of, for example, formula (XVIII) involves:

a) conversion if necessary of a quinone system for the ring *E* into the phenol system

b) conversion if necessary of a quinone system for the ring *A* into the phenol system;

c) conversion of the phenol system for the ring *A* into the methylenedioxyphenol ring;

d) formation of the bridged spiro ring system of formula (IV), (VI) or (VII) across the 1-position and 4-position in ring *B*; and

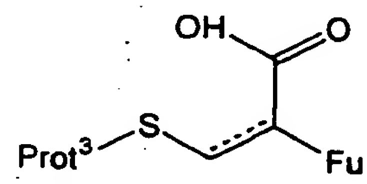
e) derivatisation as appropriate, such as acylation.

Step (a), conversion if necessary of a quinone system for the ring *E* into the phenol system, can be effected by conventional reduction procedures. A suitable reagent system is hydrogen with a palladium-carbon catalyst, though other reducing systems can be employed.

Step (b), conversion if necessary of a quinone system for the ring *A* into the phenol system is analogous to step (a), and more detail is not needed.

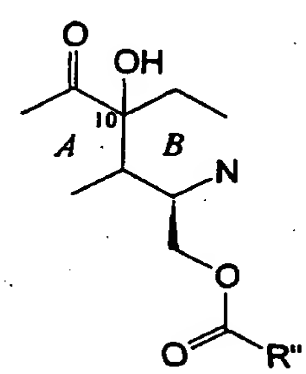
Step (c), conversion of the phenol system for the ring *A* into the methylenedioxyphenol ring, can be effected in several ways, possibly along with step (b). For example, a quinone ring A can be demethylated in the methoxy substituent at the 7-position and reduced to a dihydroquinone and trapped with a suitable electrophilic reagent such as CH_2Br_2, $BrCH_2Cl$, or a similar divalent reagent directly yielding the methylenedioxy ring system, or with a divalent reagent such as thiocarbonyldiimidazol which yields a substituted methylenedioxy ring system which can be converted to the desired ring.

Step (d) is typically effected by appropriate substitution at the 1-position with a bridging reagent that can assist formation of the desired bridge, forming an exendo quinone methide at the 4-position and allowing the methide to react with the 1-substituent to bring about the bridged structure. Preferred bridging reagents are of formula (XIX)

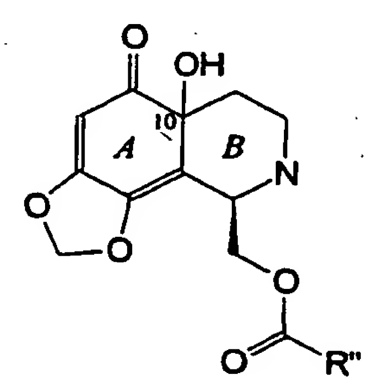


where Fu indicates a protected functional group, such as a group -$NHProt^{4a}$, $Prot^3$ is a protecting group, and the dotted line shows an optional double bond.

Suitably the methide is formed by first introducing a hydroxy group at the 10-position at the junction of rings *A* and *B* to give a partial structure of formula (XX):



or more preferably a partial structure of formula (XXI):



where the group R" is chosen for the desired group of formula (IV), (V), (VI) or (VII). For the first two such groups, the group R" typically takes the form -$CHFu$-CH_2-$SProt^3$. The protecting groups can then be removed and modified as appropriate to give the desired compound.

A typical procedure for step (d) is provided in US Patent 5,721,362 incorporated by reference. Particular reference is made to the passage at column 8, step (l) and Example 33 of the US Patent, and related passages.

Derivatisation in step (e) can include acylation, for instance with a group R^a-CO-, where R^a can be various groups such as alkyl, alkoxy, alkylene, arylalkyl, arylalkylene, amino acid acyl, or heterocyclyl, each optionally substituted with halo, cyano, nitro, carboxyalkyl, alkoxy, aryl, aryloxy, heterocyclyl, heterocyclyloxy, alkyl, amino or substituted amino. Other acylating agents include isothiocyanates, such as aryl isothiocyanates, notably phenyl isocyanate. The alkyl, alkoxy or alkylene groups of R^a suitably have 1 to 6 or 12 carbon atoms, and can be linear, branched or cyclic. Aryl groups are typically phenyl, biphenyl or naphthyl. Heterocyclyl groups can be aromatic or partially or completely unsaturated and suitably have 4 to 8 ring atoms, more preferably 5 or 6 ring atoms, with one or more heteroatoms selected from nitrogen, sulphur and oxygen.

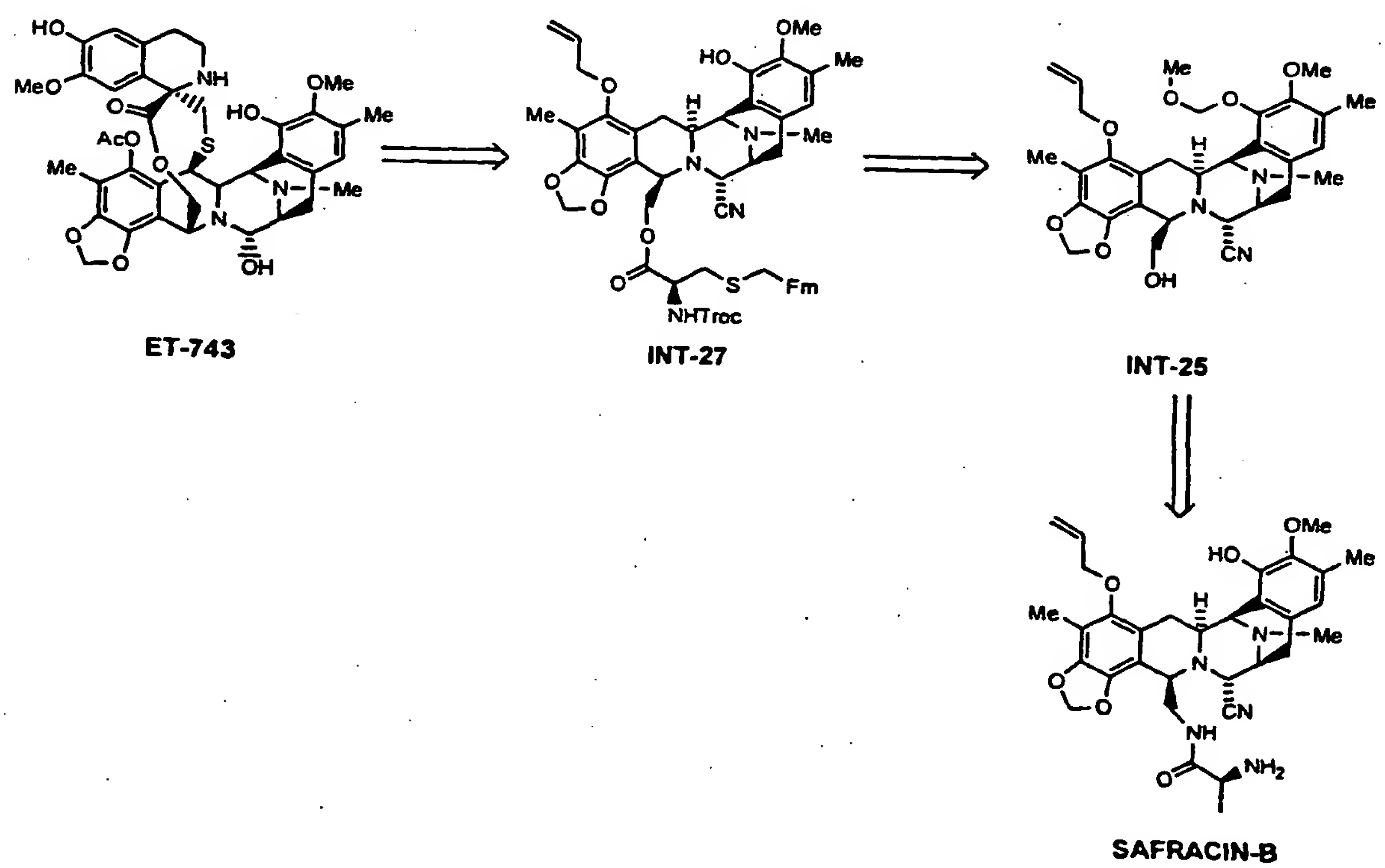
Without being exhaustive, typical R^a groups include alkyl, haloalkyl, alkoxyalkyl, haloalkoxyalkyl, arylalkylene, haloalkylarylakylene, acyl, haloacyl, arlyalkyl, alkenyl and amino acid. For example, R^a-CO- can be acetyl, trifluoroacetyl, 2,2,2-trichloroethoxycarbonyl, isovalerylcarbonyl, trans-3-(trifluoromethyl)cinnamoylcarbonyl, heptafluorobutyrylcarbonyl, decanoylcarbonyl, trans-cinnamoylcarbonyl, butyrylcarbonyl, 3-chloropropyonylcarbonyl, cinnamoylcarbonyl, 4-methylcinnamoylcarbonyl, hydrocinnamoylcarbonyl, or trans-hexenoylcarbonyl, or alanyl, arginyl, aspartyl, asparagyl, cystyl, glutamyl, glutaminyl, glycyl, histidyl, hydroxyprolyl., isoleucyl, leucyl, lysyl, methionyl, phenylalanyl, prolyl, seryl, threonyl, thyronyl, tryptophyl, tyrosyl, valyl, as well as other less common amino acid acyl

groups, as well as phthalimido and other cyclic amides. Other examples may be found among the listed protecting groups. Compounds wherein -CO-R^a is derived from an amino acid and include an amino group can themselves form acyl derivatives. Suitable N-acyl commands include dipeptides which in turn can form N-acyl derivatives.

By way of illustration, it is now feasible to transform cyanosafracin B compound of formula **2** into ET-743 resulting in a shorter and more straightforward way to make ET-743 than methods previously described.

The retrosynthetic analysis to make ET-743 using compound **29** is depicted in scheme I.

Scheme I



Following the above scheme I it is possible to obtain ET-743 in 21 linear steps. This method transforms cyanosafracin B into intermediate **25** through a sequence of reactions that involves essentially (1) removal of methoxy group placed in ring A, (2) reduction of ring A and formation of methylene-dioxy group in one pot, (3) hydrolysis of amide function placed over carbon 1, (4) transformation of the resulting amine group into hydroxyl group. Furthermore the method avoids protection and de-protection of the primary alcohol function at the position 1 in ring B of compound **25** using directly a cysteine residue **29** to form intermediate **27**. Cysteine derivative **29** is protected in the amino group with β-β-β-trichloroethoxycarbonyl protecting group in order to have compatibility with the existing allyl and MOM groups. Intermediate **27** is directly oxidized and cycled. These circumstances, together with a different de-protecting strategy in the later stages of the synthesis makes the route novel and more amenable to industrial development than the process of US 5,721,362..

The conversion of the **2**-cyano compound into Intermediate **25** usually involves the following steps (see scheme II):

formation of the protected compound of Formula **14** by reacting **2** with *tert*-butoxycarbonyl anhydride;

converting of **14** into the di-protected compound of Formula **15** by reacting with bromomethylmethyl ether and diisopropylethylamine in acetonitrile;

selectively elimination of the methoxy group of the quinone system in **15** to obtain the compound of Formula **16** by reacting with a methanolic solution of sodium hydroxide;

transforming of **16** into the methylene-dioxy compound of Formula **18** by employing the next preferred sequence: (1) quinone group of compound **16** is reduced with 10% Pd/C under hydrogen atmosphere; (2) the hydroquinone intermediate is converted into the methylenedioxy compound of Formula **17** by reacting with bromochloromethane and caesium carbonate under hydrogen atmosphere; (3) **17** is transformed into the compound of Formula **18** by protecting the free hydroxyl group as a OCH_2R group. This reaction is carried out with $BrCH_2R$ and caesium carbonate, where R can be aryl, $CH=CH_2$, OR^- etc.

elimination of the *tert*-butoxycarbonyl and the methyloxymethyl protecting groups of **18** to afford the compound of Formula **19** by reacting with a solution of HCl in dioxane. Also this reaction is achieved by mixing **18** with a solution of trifluoroacetic acid in dichloromethane;

formation of the thiourea compound of Formula **20** by reacting **19** with phenylisothiocyanate;

converting compound of Formula **20** into the amine compound of Formula **21** by reacting with a solution of hydrogen chloride in dioxane;

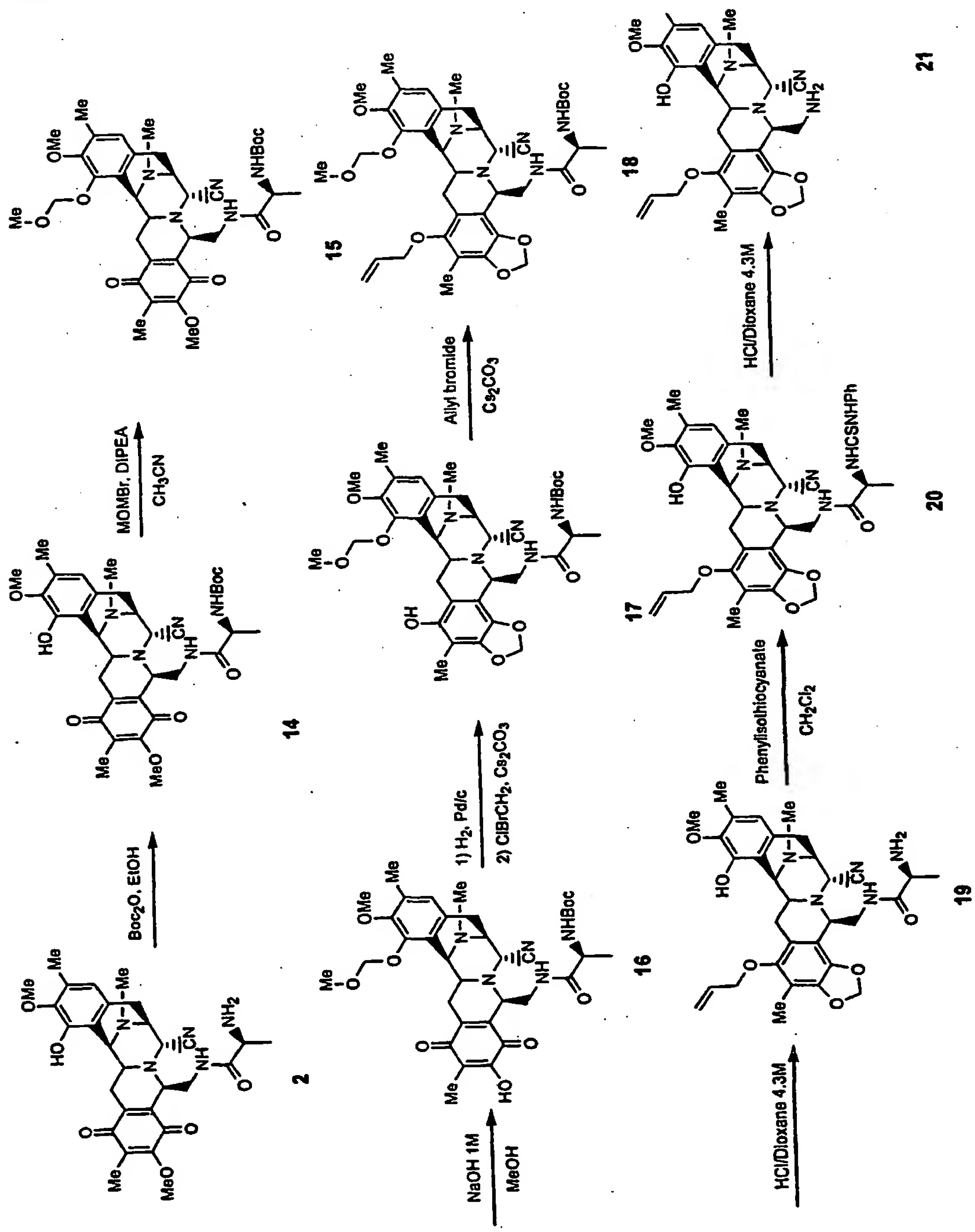
transforming compound of Formula **21** into the *N*-Troc derivative **22** by reacting with trichloroethyl chloroformate and pyridine;

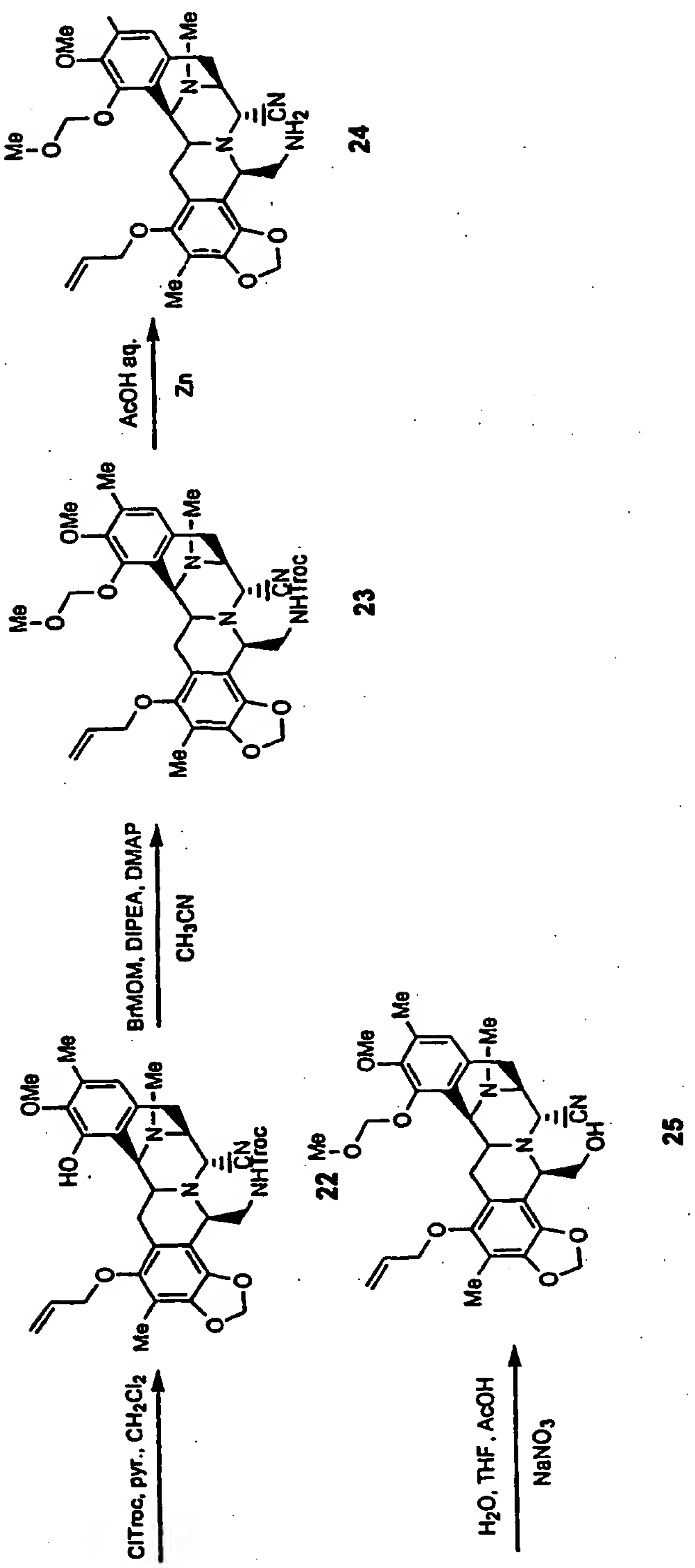
formation of the protected hydroxy compound of Formula **23** by reacting **22** with bromomethylmethyl ether and diisopropylethylamine;

transforming compound of Formula **23** into the *N*-H derivative **24** by reacting with acetic acid and zinc;

conversion of compound of Formula **24** into the hydroxy compound of Formula **25** by reaction with sodium nitrite in acetic acid. Alternatively, it can be used nitrogen tetroxide in a mixture of acetic acid and acetonitrile followed by treatment with sodium hydroxide. Also, it can be used sodium nitrite in a mixture of acetic anhydride-acetic acid, followed by treatment with sodium hydroxide.

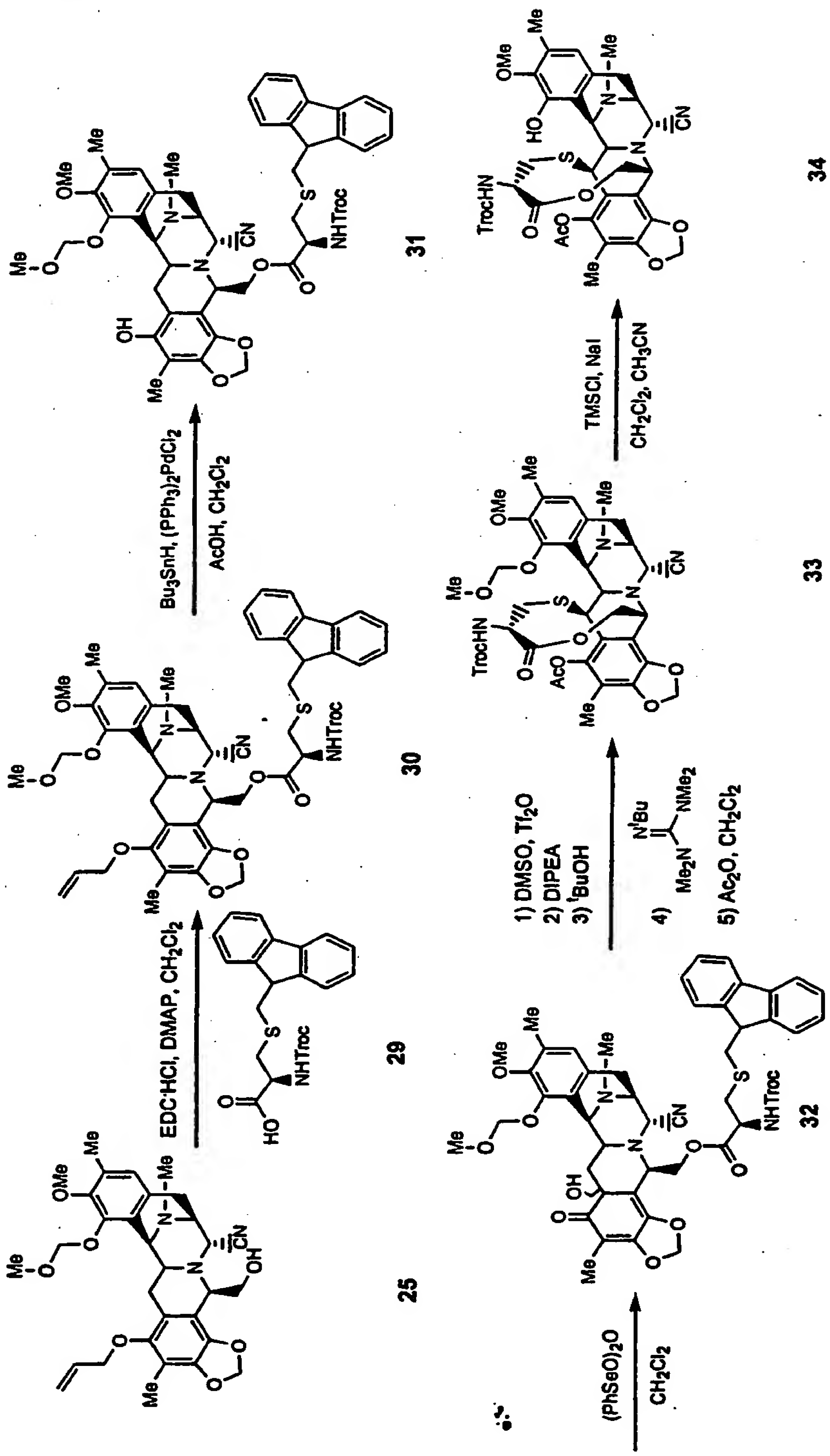
Scheme II

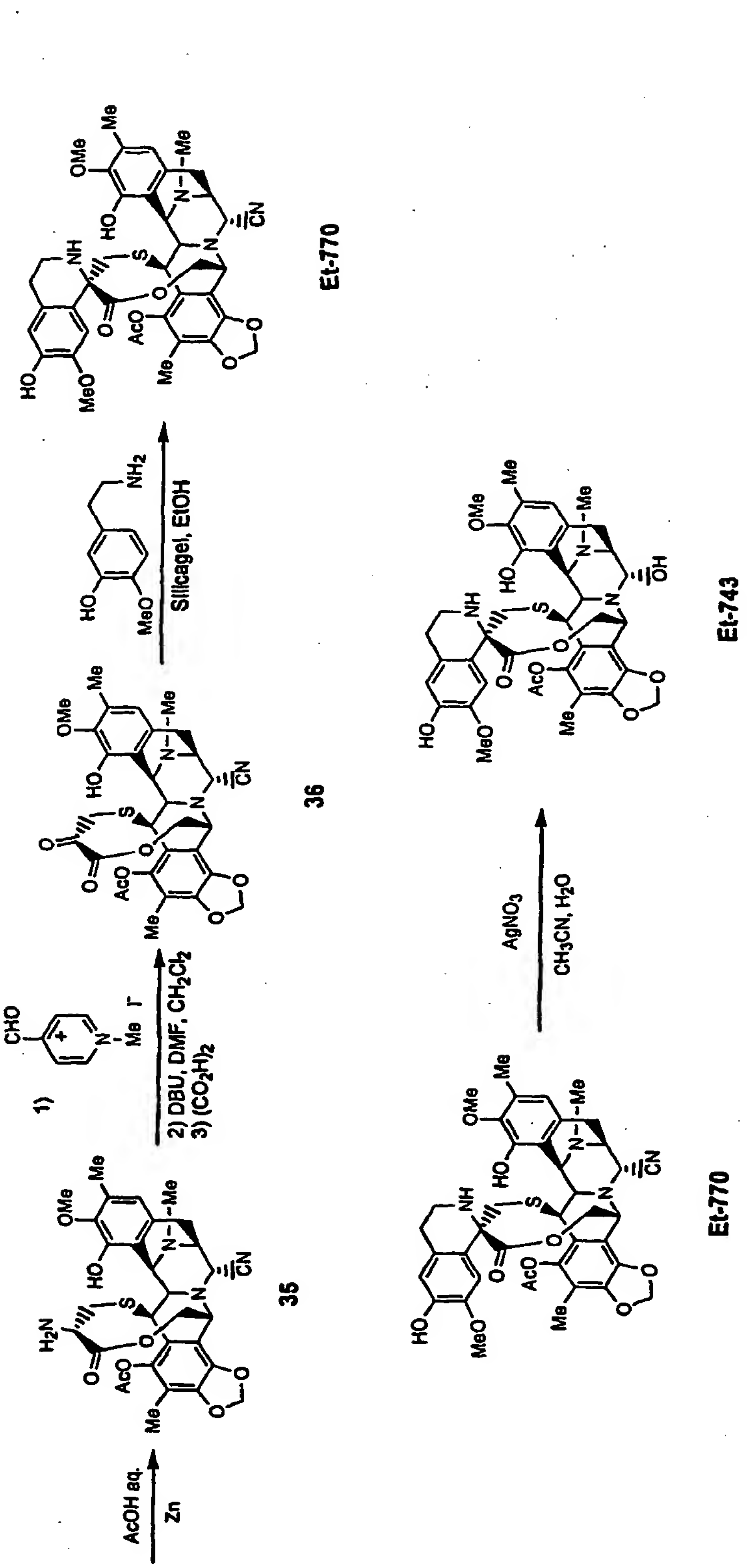



ClTroc, pyr., CH2Cl2
BrMOM, DIPEA, DMAP
CH3CN
AcOH aq.
Zn
H2O, THF, AcOH
NaNO3
22
23
24
25

The conversion of the Intermediate **25** compound into ET-743 using cysteine derivative **29** usually involves the following steps (see scheme III):

Scheme III




AcOH aq.
Zn
35
1)
2) DBU, DMF, CH_2Cl_2
3) $(CO_2H)_2$
36
Silicagel, EtOH
Et-770
$AgNO_3$
CH_3CN, H_2O
Et-770
Et-743

transforming compound of formula **24** into the derivative **30** by protecting the primary hydroxyl function with (S)-N-2,2,2-tricloroethoxycarbonyl-S-(9H-fluoren-9-ylmethyl)cysteine **29**;

converting the protected compound of formula **30** into the phenol derivative **31** by cleavage of the allyl group with tributyltin hydride and dichloropalladium-bis (triphenylphosphine);

transforming the phenol compound of Formula **31** into compound of formula **32** by oxidation with benzeneseleninic anhydride at low temperature;

transforming the hydroxy compound of formula **32** into the lactone **33** by the following sequence: (1) Reacting compound of formula **32** with 2 eq. of triflic anhydride and 5 eq. of DMSO. (2) followed by reaction with 8 eq. of diisopropylethylamine. (3) followed by reaction with 4 eq of t-butyl alcohol (4) followed by reaction with 7 eq of 2-*tert*-Butyl-1,1,3,3,tetramethylguanidine (5) followed by reaction with 10 eq of acetic anhydride;

transforming the lactone compound **33** into hydroxyl compound **34** by removal of MOM protecting group with TMSI;

cleaving the N-trichloroethoxycarbonyl group of the compound of formula **34** into compound **35** by reaction with Zn/AcOH;

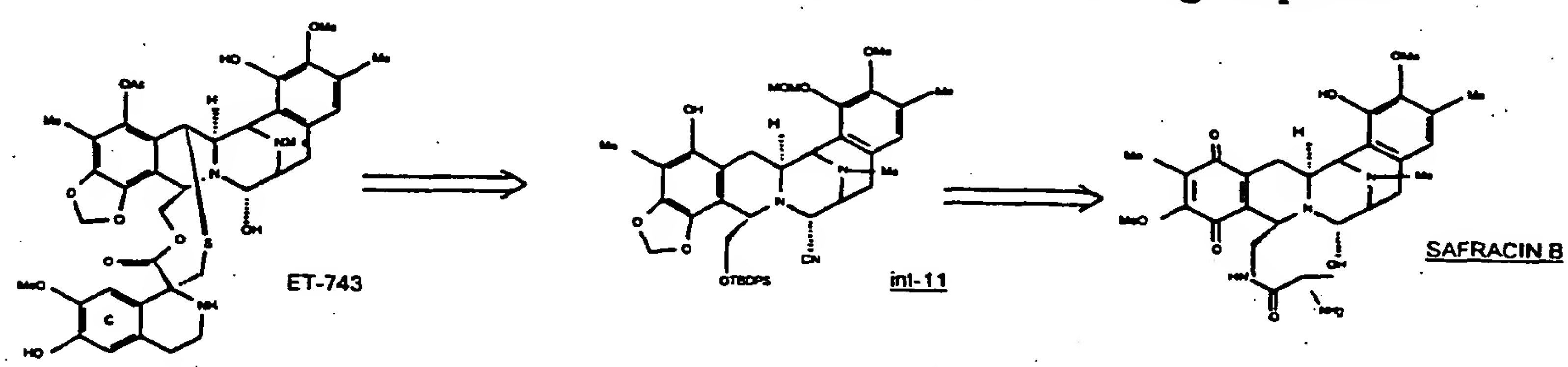
transforming the amino compound **35** into the corresponding α-keto lactone compound **36** by reaction with N-methyl pyridinium carboxaldehyde chloride followed by DBU;

forming **ET-770** by reacting compound of Formula **36** with 3-hydroxy-4-methoxyphenylethylamine;

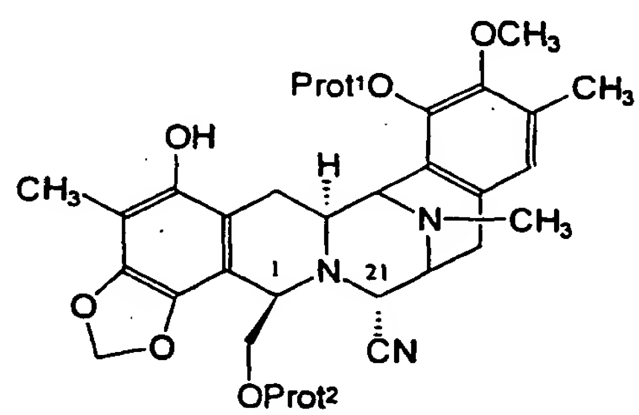
transforming **ET-770** into **ET-743** by reaction with silver nitrate in a mixture of AcN/H_2O.

FORMATION OFINTERMEDIATE11 AND RELATED INTERMEDIATES

The retrosynthetic analysis is described in the following sequence.



In the present invention, a key class of intermediate includes Intermediate 11 and has the general formula (XXI):



where Prot[1] and Prot[2] are hydroxy protecting groups, preferably different. For Intermediate 11 itself, the group Prot[1] is a methoxymethyl group, and Prot[2] is a t-butyldiphenylsilyl group.

The conversion of the 21-cyano compound to Intermediate 11 or a related intermediate of formula (XXI) usually involves the following steps:

a) conversion if necessary of a quinone system for the ring *E* into the phenol system

b) formation of the -OProt[1] group at the 18-position, in ring *E*;

c) formation of the $-CH_2-OProt^2$ group at the 1-position, in ring *B*; and

d) conversion if necessary of a quinone system for the ring *A* into the phenol system;

e) conversion of the phenol system for the ring *A* into the methylenedioxyphenol ring.

Step (b), formation of the $-OProt^1$ group at the 18-position in ring *E*, is a typical protection reaction for a phenol group, and no special comments need to be made. Appropriate conditions are chosen depending on the nature of the protecting group. The other steps are similar to the other reactions.

Step (b), formation of the $-CH_2-OProt^2$ group at the 1-position in ring *B*, is normally carried out by forming a group $-CH_2NH_2$ at the 1-position and then converting the amine function to a hydroxy function and protecting. Thus, where the starting material has a group R^1 which is $-CH_2-NH-CO-CR^{25a}R^{25b}R^{25c}$ then it is matter of removing the N-acyl group. Where the starting material has a group R^1 which is $-CH_2-O-CO-R$ then no change may be needed for an ecteinascidin product where the substituent R^1 is the same. For other products, it is matter of removing the O-acyl group. Various procedures are available for such de-acylations. In one variation, the deacylation and conversion to a hydroxy function are performed in one step. Thereafter, the hydroxy group can be acylated or otherwise converted to give the appropriate R^1 group.

U.S. Patent N° 5,721,362 describe synthetic methods to make ET-743 through a long multistep synthesis. One of the Intermediates of this synthesis is Intermediate 11. Using cyanosafracin B as starting material it is possible to reach Intermediate 11 providing a much

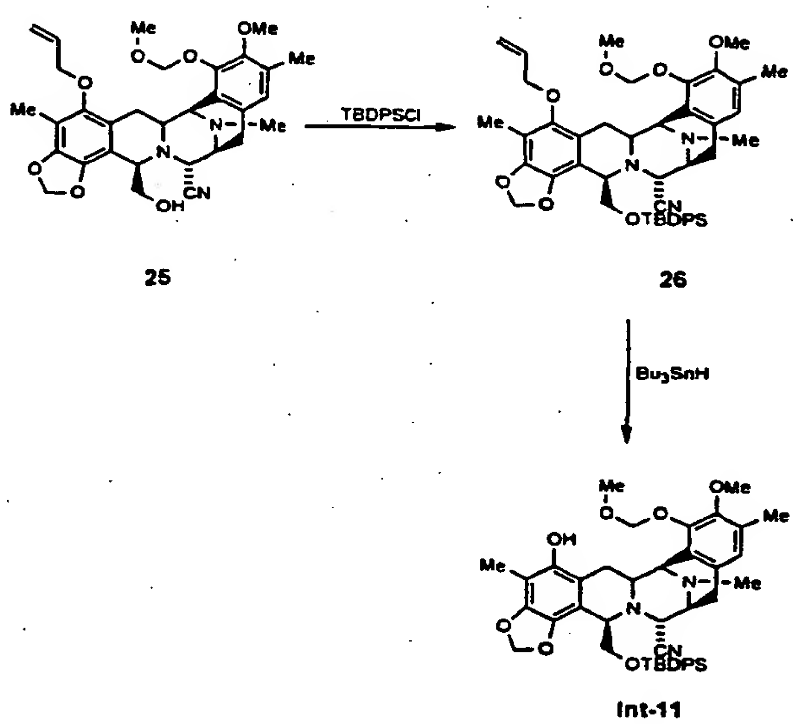
shorter way to make such Intermediate and therefor improving the method to make ET-743

Cyanosafracin B can be converted into Intermediate **25** by the methods described above. From Intermediate **25** is possible to reach Intermediate 11 using the following steps, see scheme VII.

formation of the protected hydroxy compound of Formula **26** by reacting **25** with *tert*-butyldiphenylsilyl chloride in the presence of a base;

final cleavage of the allyl group with tributyltin hydride and dichloropalladium-bis (triphenylphosphine) in **26** that leads to the formation of the intermediate **11**.

Scheme VII



One embodiment of the synthetic process of the present invention to transform safracin B into intermediate **11** is a modification and extension of Scheme VIII and comprises the sequential steps of: stereospecifically converting the compound Safracin B to the compound of Formula **2** by selective replacement of OH by CN by reacting with KCN in acid media;

forming the thiourea compound of Formula **3** by reacting compound of Formula **2** with phenyl isothiocyanate;

converting the thiourea compound of Formula **3** into the acetamide of Formula **5** by an hydrolysis in acid media followed by addition of acetic anhydride; The intermediate amine compound of Formula **4** can be isolated by quenching the hydrolysis in acid media with sodium bicarbonate, but this intermediate is highly unstable, and is transformed quickly into a five member cyclic imine, named compound **6**;

forming the protected compound of Formula **7** by reacting with bromomethylmethyl ether and diisopropylethylamine in dichloromethane;

selectively de-methylating the methoxy group of the quinone system of compound of Formula **7** into the compound of Formula **8** by reacting with methanolic solution of sodium hydroxide;

transforming the compound of Formula **8** into methylenedioxy-compound of Formula **9** by the preferred following sequence: (1) quinone group of compound **8** is reduced with 10% Pd/C under hydrogen atmosphere; (2) the hydroquinone intermediate is converted into the methylene-dioxy compound of Formula **9** by reacting with bromochloromethane and cesium carbonate under hydrogen atmosphere; (3) compound of Formula **9** is transformed into compound of Formula **10** by protecting the free hydroxyl group as a OCH_2R group, by reacting with $BrCH_2R$ and cesium carbonate, where R can be aryl, $CH=CH_2$, OR' etc.;

converting the acetamide group of compound of Formula **10** into the corresponding hydroxyl group of Formula **25** by reaction with nitrogen tetroxide in a mixture of acetic acid and acetic acetate followed by treatment with sodium hydroxide; alternatively can be used sodium nitrite in a mixture of acetic anhydride acetic acid, followed by treatment with sodium hydroxide; alternatively the acetamide group of compound of Formula **10** can be converted into the primary amine

group by reacting with hydrazine or with Boc_2O, DMAP followed by hydrazine; such primary amine can be converted into the corresponding hydroxyl group (compound of Formula **25**) by an oxidative conversion of the primary amine into the corresponding aldehyde with 4-formyl-1-methylpyridinium benzenesulphonate or other pyridinium ion, followed by DBU or other base treatment and further hydrolization, and followed by the reduction of the aldehyde to the corresponding hydroxyl group with lithium aluminium hydride or other reducing agent;
forming the protected compound of Formula **26** by reacting with t-butyldiphenylsilyl chloride and dimethylaminopyridine in dichloromethane (Scheme VII);
transforming the silylated compound of Formula **26** into the intermediate **11** by deprotection of the OCH_2R protecting group, by reacting under reductive conditions or acid conditions. Typical procedures are with palladium black under hydrogen atmosphere, or aqueous TFA, or tributyltin hydride and dichlorobis (triphenylphosphine palladium).

Scheme VIII

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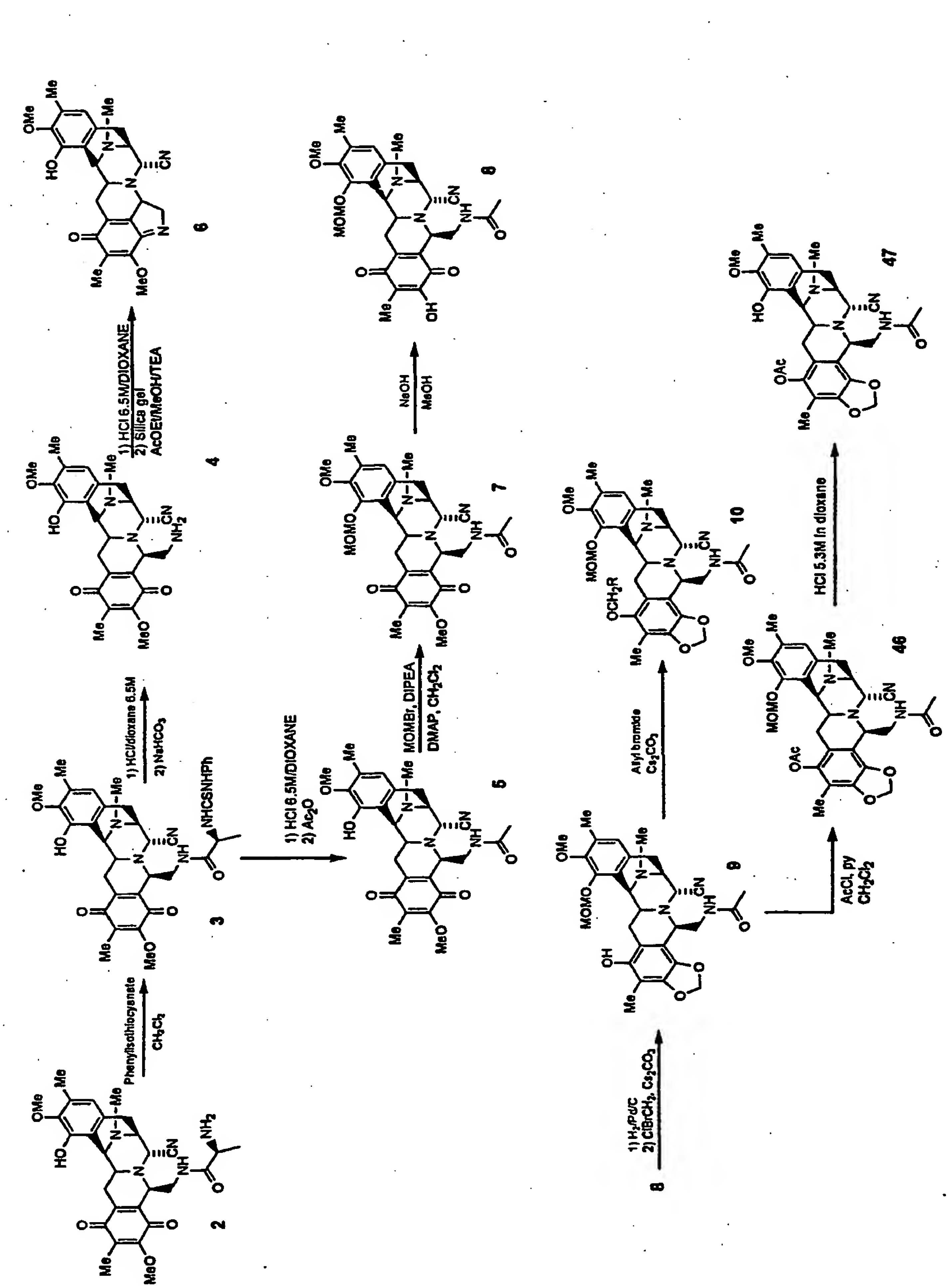

Phenylisothiocyanate
CH_2Cl_2
1) HCl/dioxane 6.5M
2) $NaHCO_3$
1) HCl 6.5M/DIOXANE
2) Silica gel
AcOEt/MeOH/TEA
1) HCl 6.5M/DIOXANE
2) Ac_2O
MOMBr, DIPEA
DMAP, CH_2Cl_2
NaOH
MeOH
1) H_2/Pd/C
2) $ClBrCH_2$, Cs_2CO_3
Allyl bromide
Cs_2CO_3
AcCl, py
CH_2Cl_2
HCl 5.3M in dioxane

In yet another preferred modification, the cyano compound of Formula **2** can be transformed into Intermediate **11** using an extension of the scheme II, involving the further steps of.

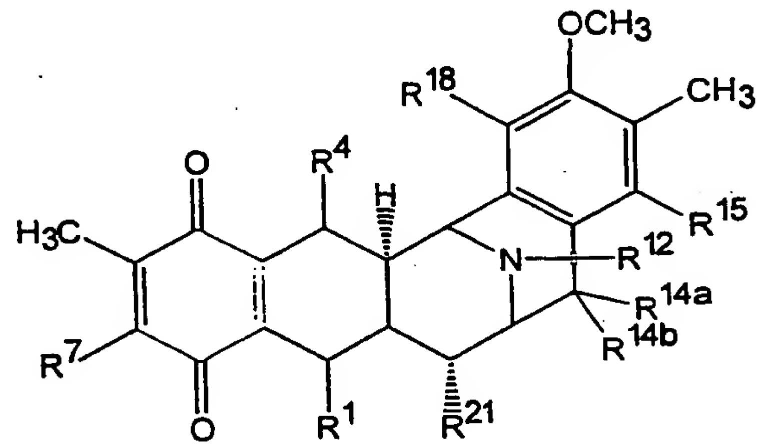
formation of the protected hydroxy compound of Formula **26** by reacting **25** with *tert*-butyldiphenylsilyl chloride in the presence of a base;

final cleavage of the allyl group with tributyltin hydride and dichloropalladium-bis (triphenylphosphine) in **26** that leads to the formation of the intermediate **11.**

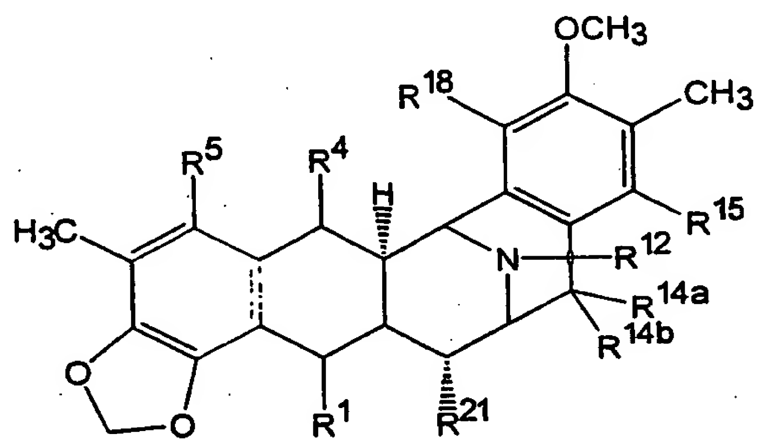
Thus, by these and other routes, it is possible to transform cyanosafracin B into a number of intermediates and derivatives with potential antitumor therapeutic activity. These intermediates can be made starting from already described compounds, or using alternative routes.

NOVEL INTERMEDIATE COMPOUNDS

In the light of the preceding explanations, it can be seen that the present invention provides novel intermediate compounds. Depending on ring A, the intermediates are of formula (XXIIa):



or of formula (XXIIb):

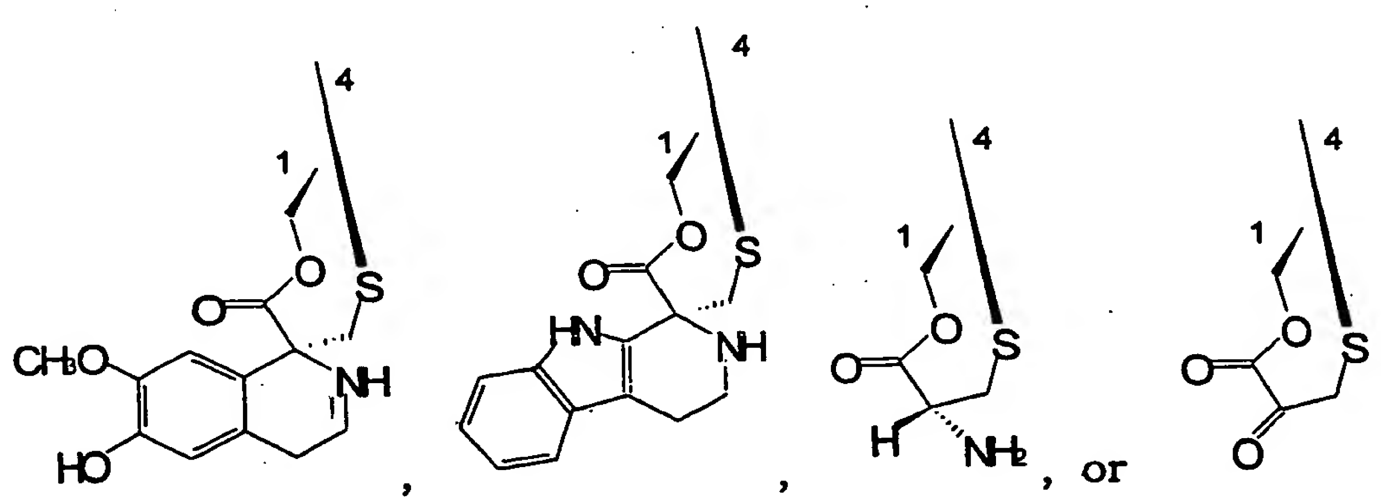


where:

R^1 is $-CH_2NH_2$ or $-CH_2OH$, or a protected or derivatised version of such a group and R^4 is -H;

or

R^{1a} and R^4 together form a group of formula (IV), (V), (VI) or (VII):

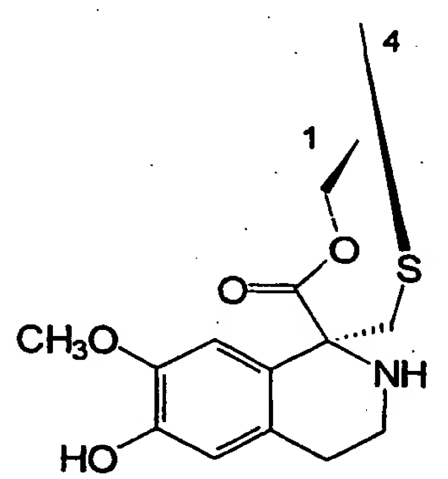


R^5 is -OH or a protected or derivatised version of such a group;
R^{14a} and R^{14b} are both -H or one is -H and the other is -OH or a protected or derivatised version of such a group, $-OCH_3$ or $-OCH_2CH_3$, or R^{14a} and R^{14b} together form a keto group;
R^{12} is -H-, $-CH_3$- or $-CH_2CH_3$-;
R^{15} is –H, -OH or a protected or derivatised version of such a group; and
R^{18} is -OH or a protected or derivatised version of such a group.

In one embodiment, preferably at least of R^1 , R^5, R^{14a}, R^{14b}, R^{15} or R^{18} is a protected or derivatised group.

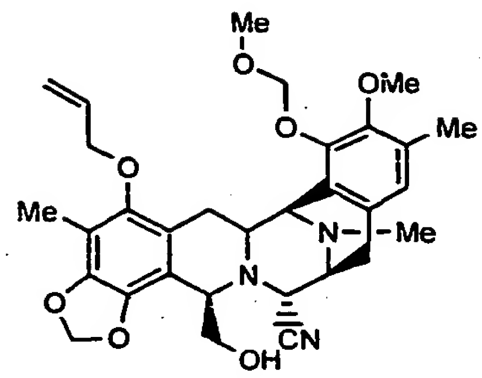
In one variation of this invention, the group R^1 is not a tert-butyldiphenylsilyl substituent and/or the group R^{18} is not a methoxymethyl group.

Preferably R^1 is $-CH_2NH_2$ or $-CH_2OH$, or a protected or derivatised version of such a group and R^4 is -H;
or
R^{1a} and R^4 together form a group:



Preferably R^{14a} and R^{14b} are both -H.

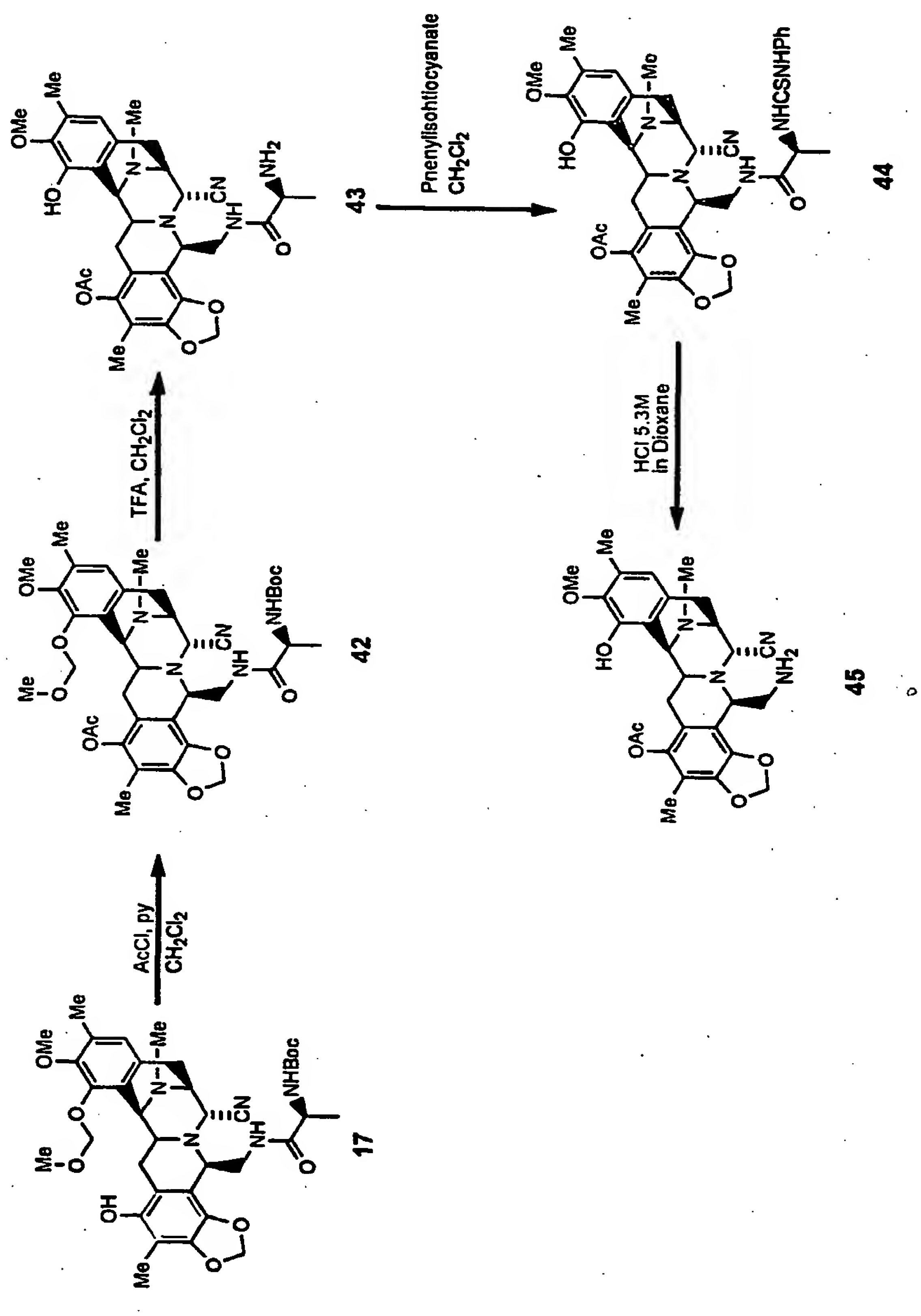
One preferred class of intermediates includes the compound which we identify as compound **25**, of formula:



25

The preferred class is thus of the general formula where the group MOM is replaced by any other protecting group.

Other preferred intermediates includes the compounds which we identify as compound **45** and **43** (Scheme IX).



Other N-acyl derivatives may readily be made from compound **45** and are an important part of this invention. Suitable acyl groups include those previously mentioned. The corresponding 21-hydroxy compounds are also useful and are among the active compounds which we have found.

NOVEL ACTIVE COMPOUNDS

We have additionally found that certain of the compounds of the invention which we initially prepared as intermediates have exceptional activity in the treatment of cancers, such as leukaemias, lung cancer, colon cancer, kidney cancer and melanoma.

Thus, the present invention provides a method of treating any mammal, notably a human, affected by cancer which comprises administering to the affected individual a therapeutically effective amount of a compound of the invention, or a pharmaceutical composition thereof.

The present invention also relates to pharmaceutical preparations, which contain as active ingredient a compound or compounds of the invention, as well as the processes for their preparation.

Examples of pharmaceutical compositions include any solid (tablets, pills, capsules, granules, etc.) or liquid (solutions, suspensions or emulsions) with suitable composition or oral, topical or parenteral administration, and they may contain the pure compound or in combination with any carrier or other pharmacologically active compounds. These compositions may need to be sterile when administered parenterally.

Administration of the compounds or compositions of the present invention may be by any suitable method, such as intravenous infusion, oral preparations, intraperitoneal and intravenous administration. We prefer that infusion times of up to 24 hours are used, more preferably 2-12 hours, with 2-6 hours most preferred. Short infusion times which

allow treatment to be carried out without an overnight stay in hospital are especially desirable. However, infusion may be 12 to 24 hours or even longer if required. Infusion may be carried out at suitable intervals of say 2 to 4 weeks. Pharmaceutical compositions containing compounds of the invention may be delivered by liposome or nanosphere encapsulation, in sustained release formulations or by other standard delivery means.

The correct dosage of the compounds will vary according to the particular formulation, the mode of application, and the particular *situs*, host and tumour being treated. Other factors like age, body weight, sex, diet, time of administration, rate of excretion, condition of the host, drug combinations, reaction sensitivities and severity of the disease shall be taken into account. Administration can be carried out continuously or periodically within the maximum tolerated dose.

The compounds and compositions of this invention may be used with other drugs to provide a combination therapy. The other drugs may form part of the same composition, or be provided as a separate composition for administration at the same time or a different time. The identity of the other drug is not particularly limited, and suitable candidates include:

a) drugs with antimitotic effects, especially those which target cytoskeletal elements, including microtubule modulators such as taxane drugs (such as taxol, paclitaxel, taxotere, docetaxel), podophylotoxins or vinca alkaloids (vincristine, vinblastine);

b) antimetabolite drugs such as 5-fluorouracil, cytarabine, gemcitabine, purine analogues such as pentostatin, methotrexate);

c) alkylating agents such as nitrogen mustards (such as cyclophosphamide or ifosphamide);

d) drugs which target DNA such as the antracycline drugs adriamycin, doxorubicin, pharmorubicin or epirubicin;

e) drugs which target topoisomerases such as etoposide;

f) hormones and hormone agonists or antagonists such as estrogens, antiestrogens (tamoxifen and related compounds) and androgens, flutamide, leuprorelin, goserelin, cyprotrone or octreotide;

g) drugs which target signal transduction in tumour cells including antibody derivatives such as herceptin;

h) alkylating drugs such as platinum drugs (cis-platin, carbonplatin, oxaliplatin, paraplatin) or nitrosoureas;

i) drugs potentially affecting metastasis of tumours such as matrix metalloproteinase inhibitors;

j) gene therapy and antisense agents;

k) antibody therapeutics;

l) other bioactive compounds of marine origin, notably the didemnins such as aplidine;

m) steroid analogues, in particular dexamethasone;

n) anti-inflammatory drugs, in particular dexamethasone; and

o) anti-emetic drugs, in particular dexamethasone.

The present invention also extends to the compounds of the invention for use in a method of treatment, and to the use of the compounds in the preparation of a composition for treatment of cancer.

CYTOTOXIC ACTIVITY

Cell Cultures. Cells were maintained in logarithmic phase of growth in Eagle's Minimum Essential Medium, with Earle's Balanced Salts, with 2.0 mM L-glutamine, with non-essential amino acids, without sodium bicarbonate (EMEM/neaa); supplemented with 10% Fetal Calf Serum (FCS), 10^{-2} M sodium bicarbonate and 0.1 g/l penicillin-G + streptomycin sulfate.

A simple screening procedure has been carried out to determine and compare the antitumour activity of these compounds, using an adapted form of the method described by Bergeron et al (1984). The tumour cell line employed have been P-388 (suspension culture of a lymphoid neoplasm from DBA/2 mouse), A-549 (monolayer culture of a human lung carcinoma), HT-29 (monolayer culture of a human colon carcinoma) and MEL-28 (monolayer culture of a human melanoma).

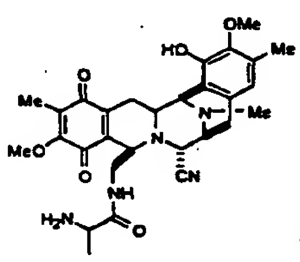
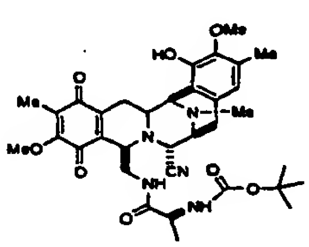
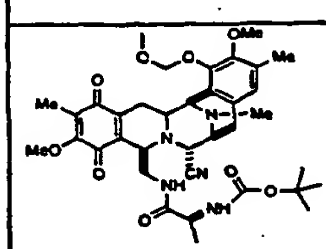
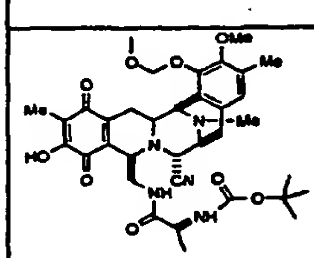
P-388 cell were seeded into 16 mm wells at $1x10^4$ cells per well in 1 ml aliquots of MEM 5FCS containing the indicated concentration of drug. A separate set of cultures without drug was seeded as control growth to ensure that cells remained in exponential phase of growth. All determinations were carried out in duplicate. After three days of incubation at 37°C, 10% CO_2 in a 98% humid atmosphere, an approximately IC_{50} was determined by comparing the growth in wells with drug to the growth in wells control.

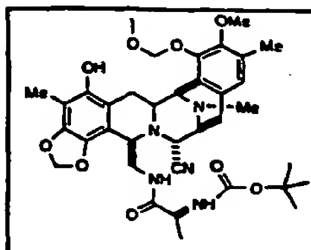
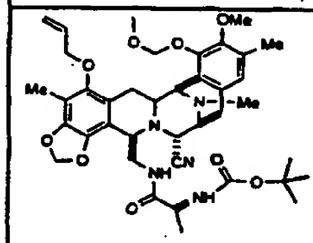
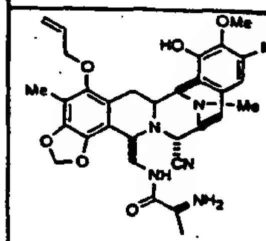
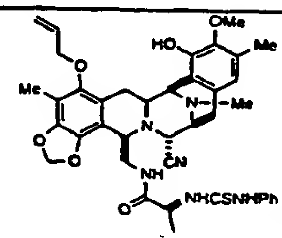
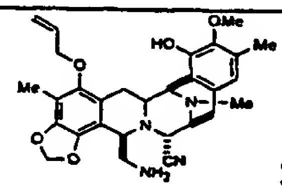
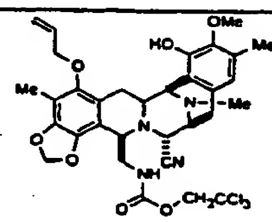
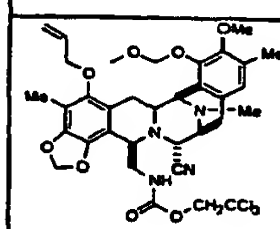
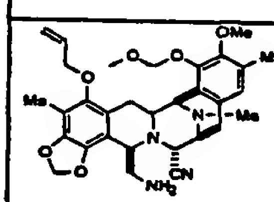
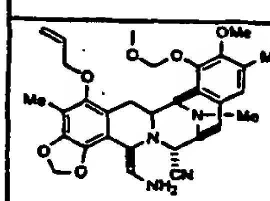
A-549, HT-29 and MEL-28 were seeded into 16 mm wells at $2x10^4$ cells per well in 1 ml aliquots of MEM 10FCS containing the indicated concentration of drug. A separate set of cultures without drug was seeded as control growth to ensure that cells remained in exponential phase of growth. All determinations were carried out in duplicate. After three days of incubation at 37°C, 10% CO_2 in a 98% humid atmosphere, the wells were stained with 0.1% Crystal Violet. An approximately IC_{50} was determined by comparing the growth in wells with drug to the growth in wells control.

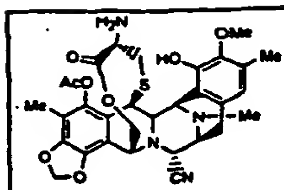
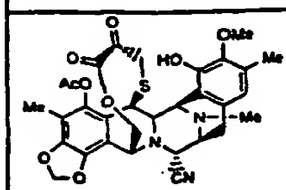
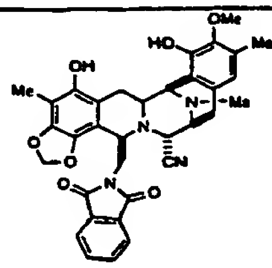
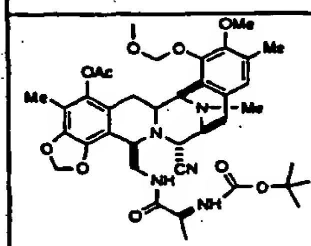
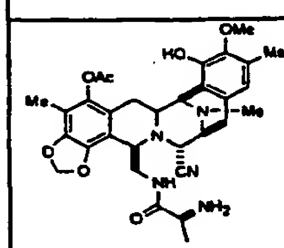
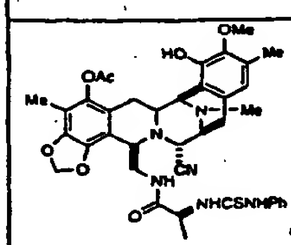
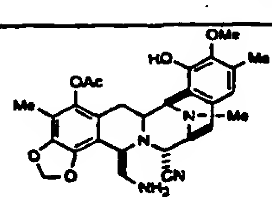
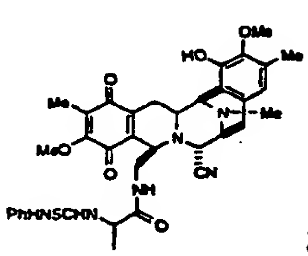
1. Raymond J. Bergeron, Paul F. Cavanaugh, Jr., Steven J. Kline. Robert G. Hughes, Jr., Gary T. Elliot and Carl W. Porter. Antineoplastic and antiherpetic activity of spermidine catecholamide iron chelators. *Biochem. Bioph. Res. Comm. 1984,* 121(3), 848-854.

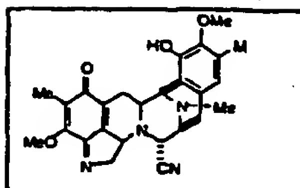
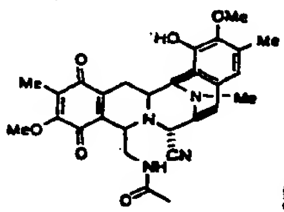
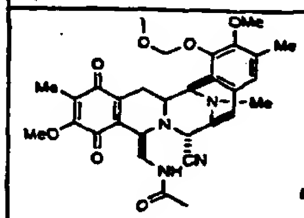
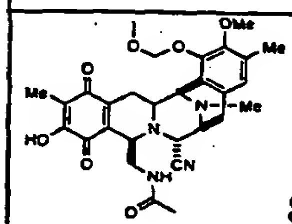
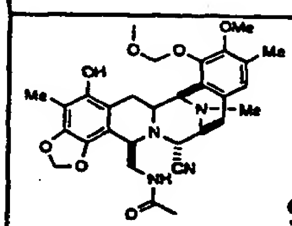
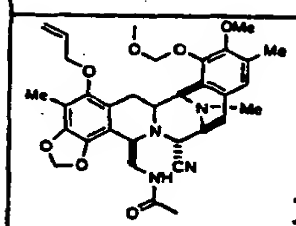
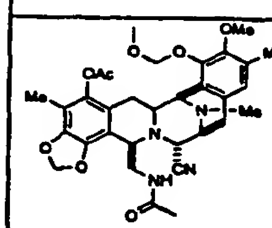
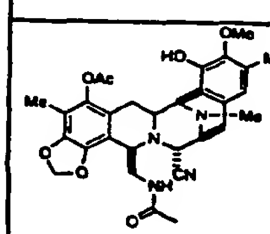
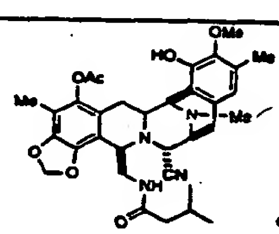
2. Alan C. Schroeder, Robert G. Hughes, Jr. and Alexander Bloch. Effects of Acyclic Pyrimidine Nucleoside Analoges. *J. Med. Chem.* 1981, 24 1078-1083.

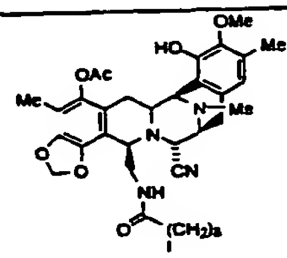
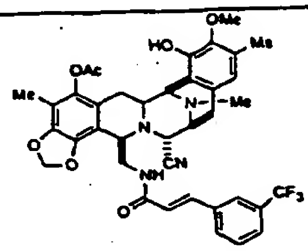
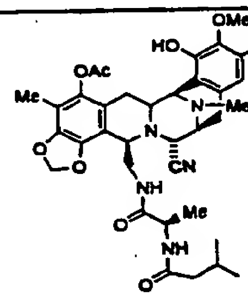
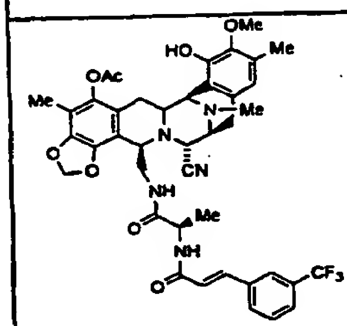
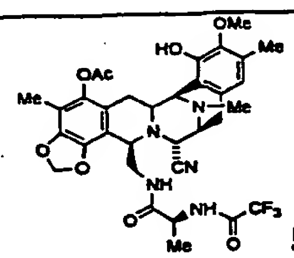
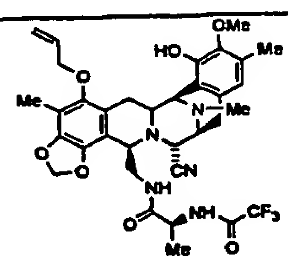
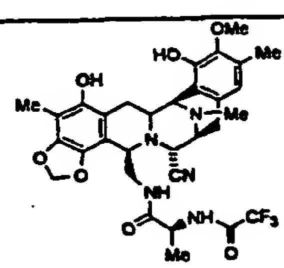
Cytotoxic activity

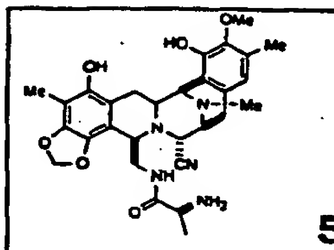
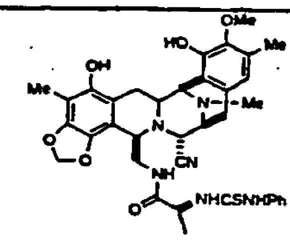
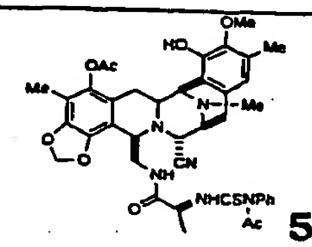
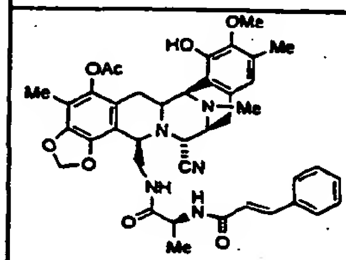
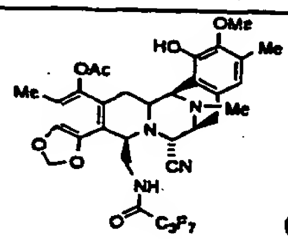
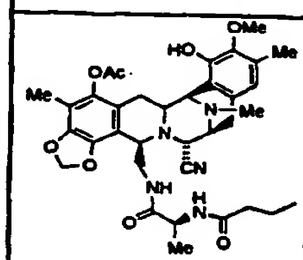
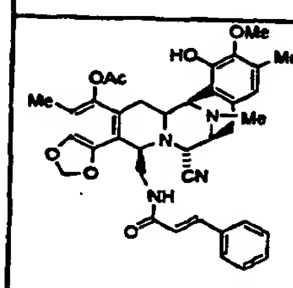
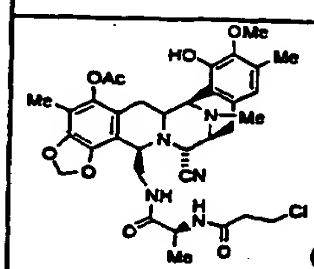
Compound	IC_{50} (μM)					
	P-388	**A-549**	**HT-29**	**MEL-28**	**CV-1**	**DU-145**
 **2**	0.009	0.018	0.018	0.018	0.023	
 **14**	0.15	>0.15	0.15	>0.15		
 **15**	1.44	1.44	1.44	1.44		
 **16**	>1.5	>1.5	>1.5	>1.5		

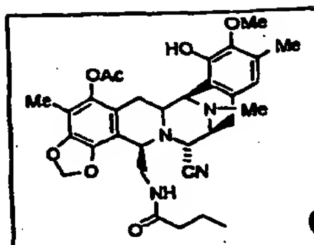
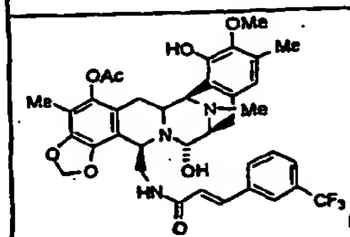
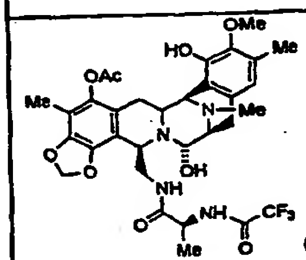
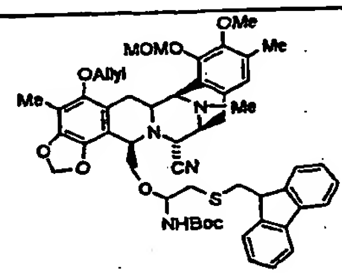
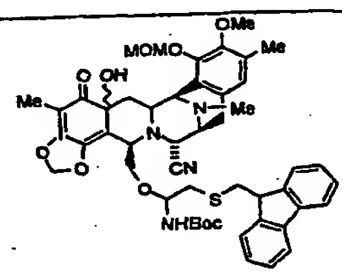
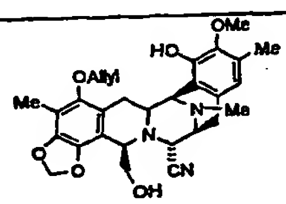
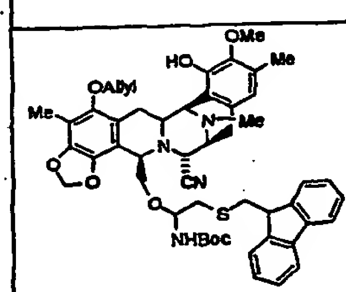
 17	1.4	1.4	1.4	1.4		
 18	0.01	0.01	0.01	0.01		
 19	0.08	0.16	0.01	0.16		
 20	0.01	0.01	0.01	0.01		
 21	0.019	0.019	0.019	0.019		
 22	0.014	0.014	0.014	0.014	0.014	0.014
 23	0.13	0.13	0.13	0.13	0.13	0.13
 24	0.18	1.8	1.8	1.8	1.8	1.8
 25	0.2	0.2	0.2	0.2		0.2

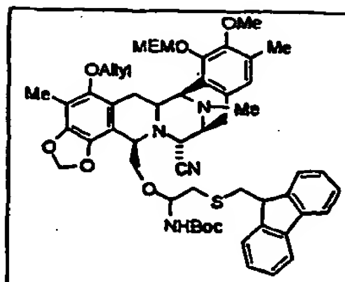
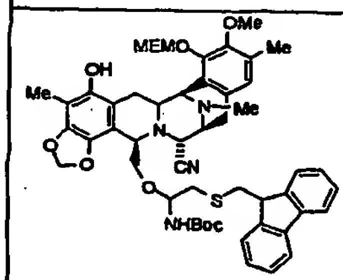
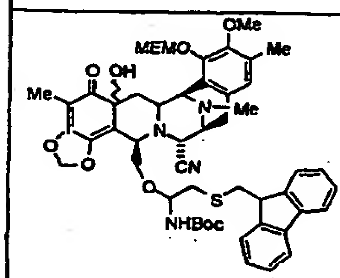
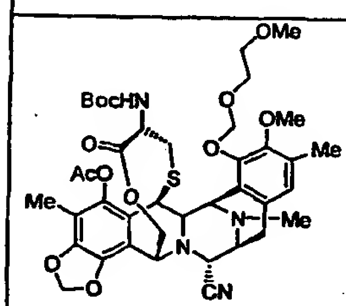
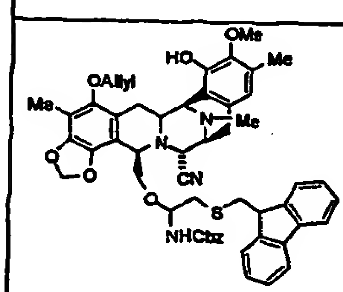
 35	0.008	0.008	0.008	0.008		
 36	0.01	0.01	0.01	0.01		
 28	0.001	0.001	0.001	0.001	0.001	0.001
 42	0.13	0.13	0.13	0.13		0.13
 43	0.008	0.016	0.008	0.008		0.016
 44	0.001	0.001	0.001	0.001		0.001
 45	0.01	0.01	0.01	0.01		0.01
 3	0.015	0.015	0.015	0.015	0.018	

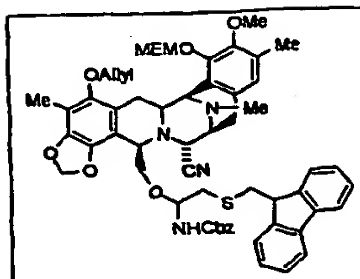
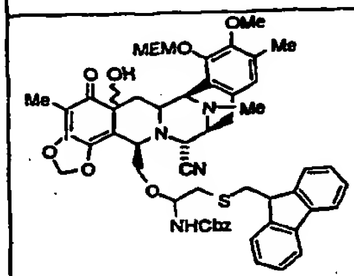
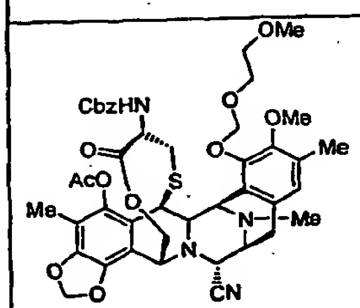
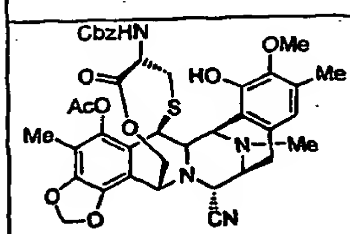
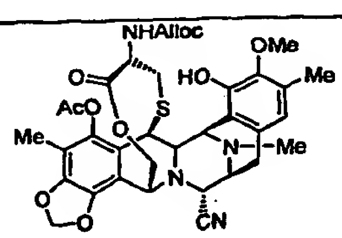
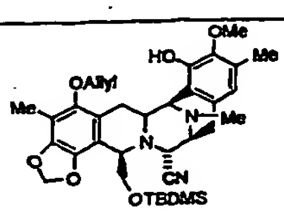
 6	2.171	2.171	2.171	2.171	2.171	
 5	0.005	0.005	0.005	0.005		
 7	0.22	0.22	0.22	0.22	0.22	
 8	>9	>18.1	>18.1	>18.1	>18.1	
 9	>1.77	>1.77	>1.77	>1.77		>1.77
 10	>1.65	>1.65	>1.65	>1.65		>1.65
 46	0.016	0.016	0.016	0.016		0.016
 47	0.001	0.001	0.001	0.001		0.001
 48	0.0008	0.001	0.0008	0.0008		0.001

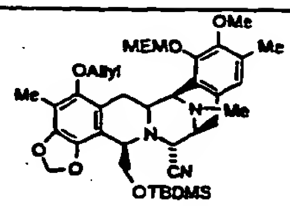
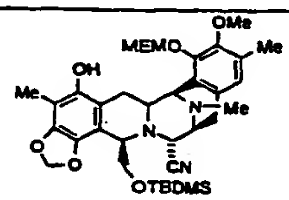
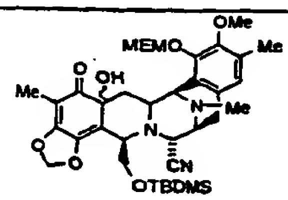
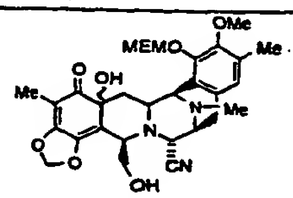
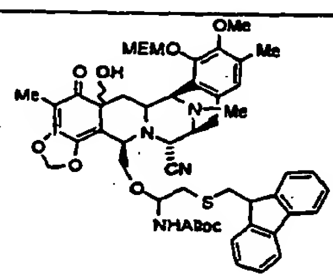
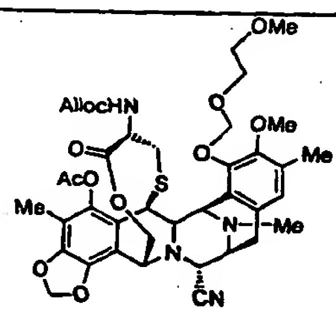
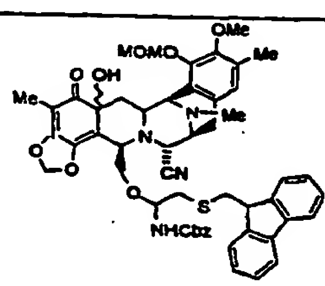
 49	0.007	0.007	0.007	0.007		0.007
 50	0.0001	0.0001	0.0001	0.0001		0.0001
 51	0.0001	0.0001	0.0001	0.0001		0.0001
 52	0.001	0.001	0.001	0.001		0.001
 53	0.0001	0.0001	0.0001	0.0001		0.0001
 54	0.001	0.001	0.001	0.001		0.001
 55	0.01	0.01	0.01	0.01		0.01

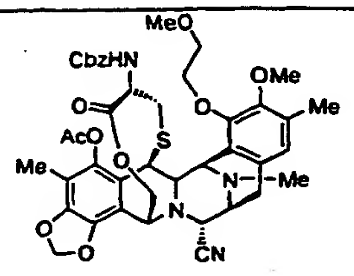
 56	0.18	0.9	0.18	0.8		0.9
 57	0.14	0.14	0.14	0.14		0.14
 58	0.001	0.001	0.001	0.001		0.001
 60 60	0.001	0.001	0.0005	0.001		0.0005
 61	0.001	0.001	0.001	0.001		0.001
 62	0.001	0.001	0.0005	0.0005		0.001
 63	0.0001	0.0001	0.0001	0.0001		0.0001
 64	0.001	0.001	0.001	0.001		0.001

 65	0.0001	0.0005	0.0001	0.0001		0.0005
 66	0.0001	0.0001	0.0001	0.0001		0.0001
 67	0.0001	0.0001	0.0001	0.0001		0.0001
 142		>1	>1			
 144		>1	>1			
 146	0.19	0.19	0.19	0.19		
 147		0.0055	0.0055			

 148		>1	>1			
 149		0.01	0.01			
 150		0.051	0.051			
 151		0.012	0.012			
 153		0.11	0.11			

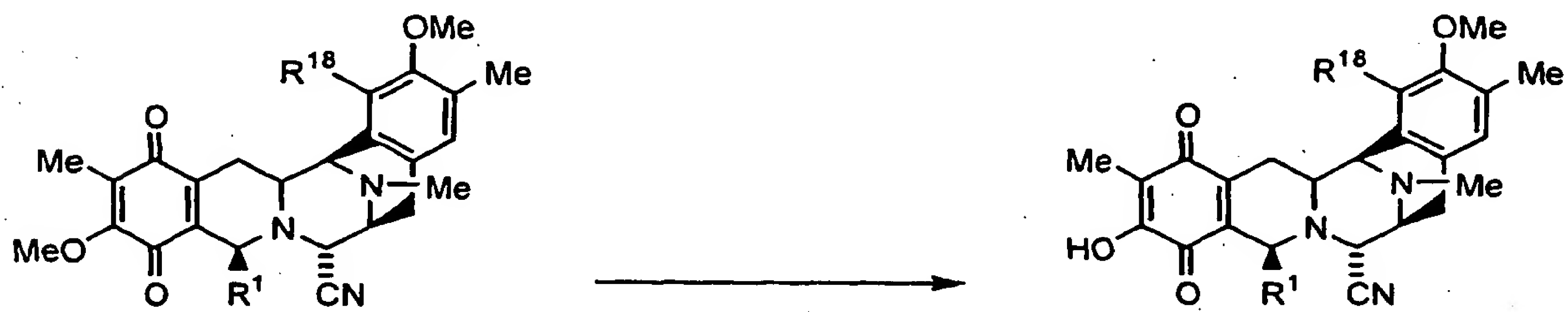
 154		>1	>1			
 156		>1	>1			
 157		0.59	0.59			
 158		0.0013	0.0013			
 164		0.00015	0.00015			
 165		>1	>1			

 166		>1	>1			
 167		>1	>1			
 168		>1	>1			
 169		>1	>1			
 170		>1	>1			
 171		0.012	0.012			
 172		>1	>1			

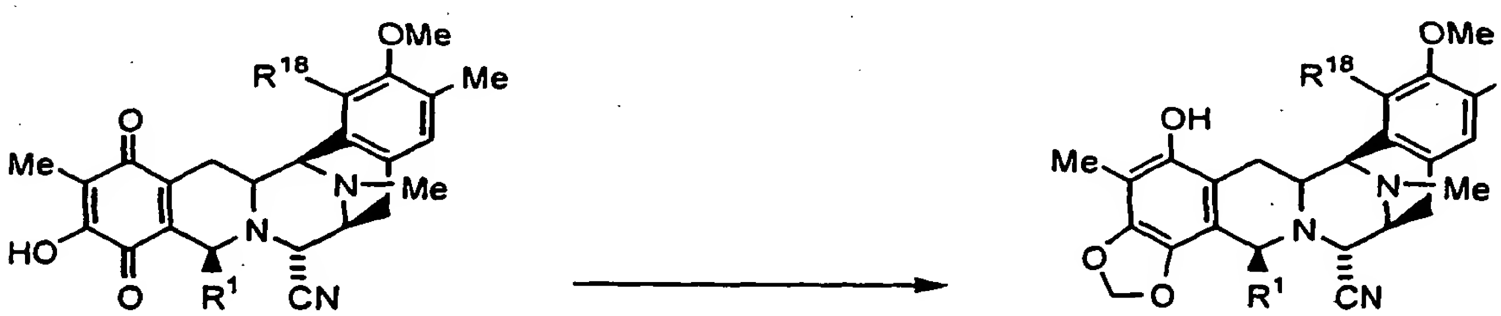
 173		0.062	0.062			

The active compounds of this invention thus include compounds with the 10-hydroxy group and the 1-labile group.

An important method of this invention includes the reaction:

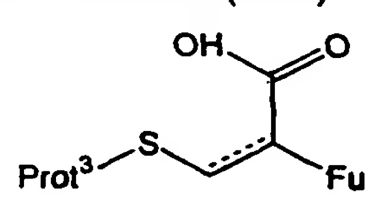


Another important method of this invention includes the reaction:



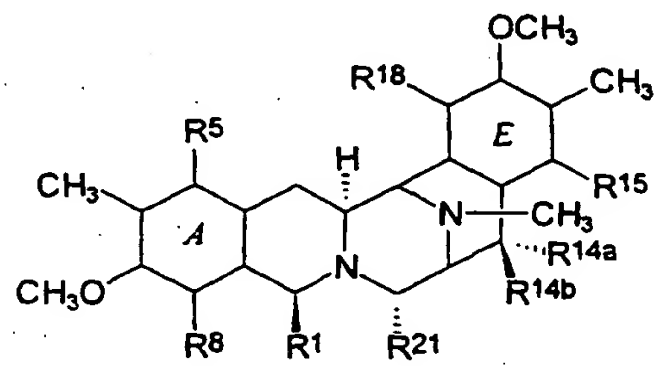
Another important method of this invention includes the reaction includes the reaction where a group R^1 is aminomethylene is converted to a hydroxymethylene group.

Another important method of this invention includes the reaction wherein a compound with a group R^1 which is hydroxymethylene is reacted with a reagent of the formula (XIX)



where Fu indicates a protected functional group, $Prot^3$ is a protecting group, and the dotted line shows an optional double bond.

Another important method of this invention includes the reaction for preparing a 21-cyano compound of formula (XVI) which comprises reacting a compound of formula (XV):



where R^1, R^5, R^8, R^{14a}, R^{14b}, R^{15} and R^{18} are as defined and R^{21} is a hydroxy group, with a source of cyanide ion, to give the desired 21-cyano compound.

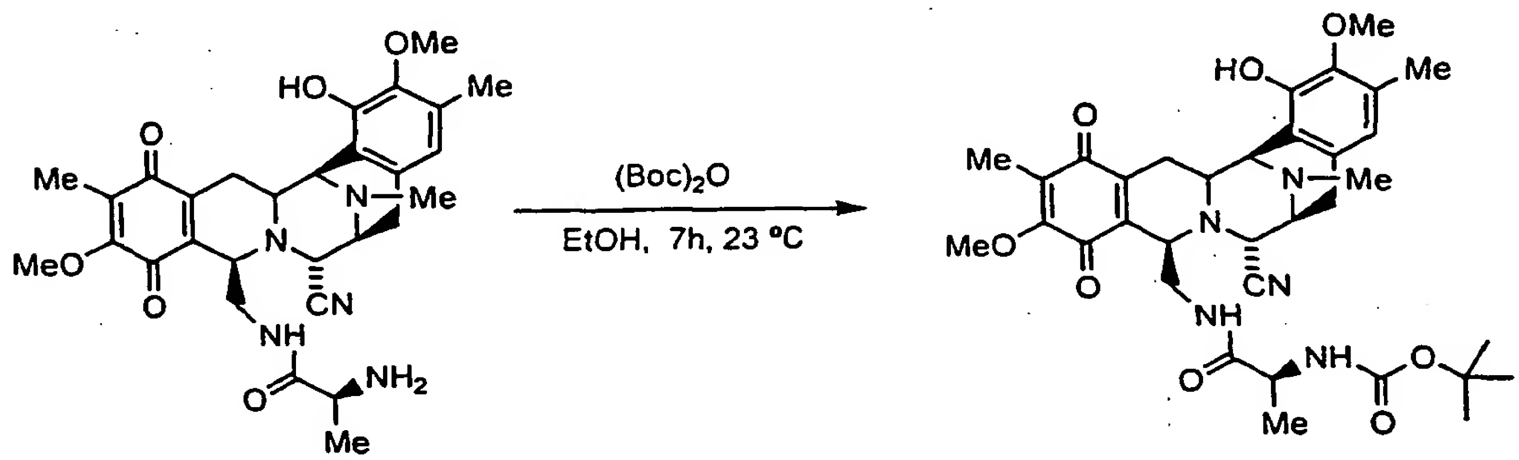
In addition, processes using other nucleophile-containing compounds, to produce similar compounds of formula (XVI) wherein the 21-position is protected by another nucleophilic group, a 21-Nuc group, are also envisaged. For example, a 21-Nuc compound of formula (XVI) with an alkylamino substituent at the 21-position can be produced by reacting the compound of formula (XV) wherein R^{21} is a hydroxy group with a suitable alkylamine. A 21-Nuc compound of formula (XVI) with an alkylthio substituent at the 21-position can also be produced by reacting the compound of formula (XV) wherein R^{21} is a hydroxy group with a suitable alkanethiol. Alternatively, a 21-Nuc compound of formula (XVI) with an □-carbonylalkyl substituent at the 21-position can be produced by reacting the compound of formula (XV) wherein R^{21} is a hydroxy group with a suitable carbonyl compound, typically in the presence of a base. Other synthetic routes are available for other 21-Nuc compounds.

Another important reaction of this invention involves treatment of a 21-cyano product of this invention to form a 21-hydroxy compound. Such compounds have interesting *in vivo* properties.

EXAMPLES

The present invention is illustrated by the following examples.

Example 1



2 **14**

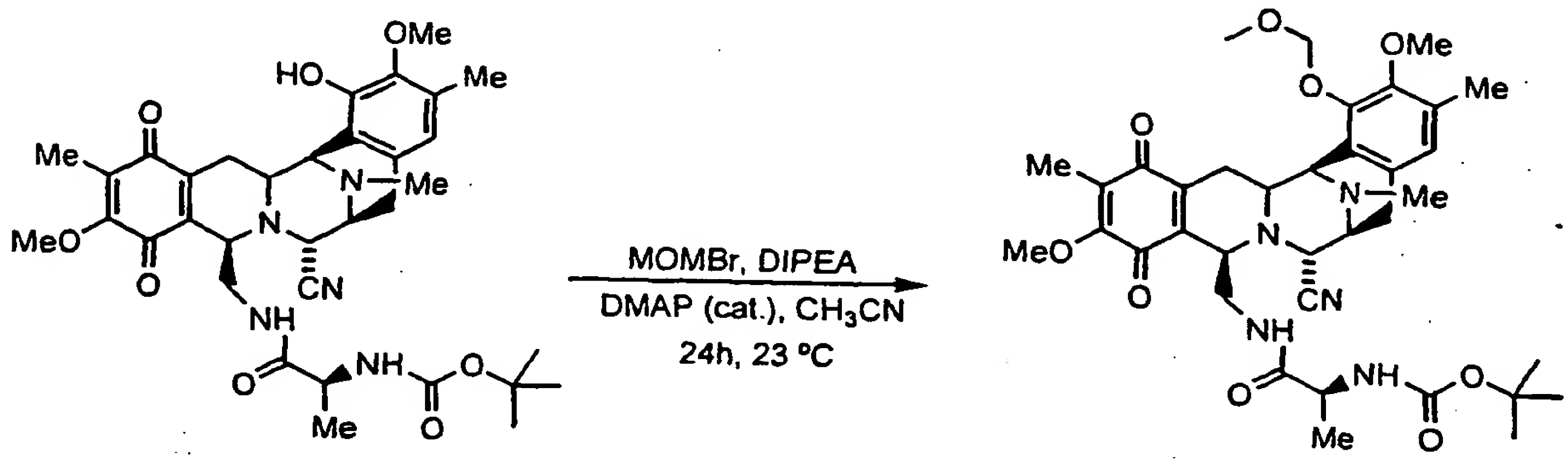
To a solution of **2** (21.53 g, 39.17 mmol) in ethanol (200 ml), *tert*-butoxycarbonyl anhydride (7.7 g, 35.25 mmol) was added and the mixture was stirred for 7 h at 23 °C. Then, the reaction was concentrated *in vacuo* and the residue was purified by flash column chromatography (SiO_2, hexane:ethyl acetate 6:4) to give **14** (20.6 g, 81 %) as a yellow solid.

Rf: 0.52 (ethyl acetate:$CHCl_3$ 5:2).
^{1}H NMR (300 MHz, $CDCl_3$): δ 6.49 (s, 1H), 6. 32 (bs, 1H), 5.26 (bs, 1H), 4.60 (bs, 1H), 4.14 (d, *J*= 2.4 Hz, 1H), 4.05 (d, *J*= 2.4 Hz, 1H), 3.94 (s, 3H), 3.81 (d, *J*= 4.8 Hz, 1H), 3.7 (s, 3H), 3.34 (br d, *J*= 7.2 Hz, 1H), 3.18-3.00 (m, 5H), 2.44 (d, *J*= 18.3 Hz, 1H), 2.29 (s, 3H), 2.24 (s, 3H), 1.82 (s, 3H), 1.80-1.65 (m, 1H), 1.48 (s, 9H), 0.86 (d, *J*= 5.7 Hz, 3H)

^{13}C NMR (75 MHz, $CDCl_3$): δ 185.5, 180.8, 172.7, 155.9, 154.5, 147.3, 143.3, 141.5, 135.3, 130.4, 129.2, 127.5, 120.2, 117.4, 116.9, 80.2, 60.7, 60.3, 58.5, 55.9, 55.8, 54.9, 54.4, 50.0, 41.6, 40.3, 28.0, 25.3, 24.0, 18.1, 15.6, 8.5.

ESI-MS m/z: Calcd. for $C_{34}H_{43}N_5O_8$: 649.7. Found $(M+H)^+$: 650.3.

Example 2



14 15

To a stirred solution of **14** (20.6 g, 31.75 mmol) in CH_3CN (159 ml), diisopropylethylamine (82.96 ml, 476.2 mmol), methoxymethylene bromide (25.9 ml, 317.5 mmol) and dimethylaminopyridine (155 mg, 1.27 mmol) were added at 0 °C. The mixture was stirred at 23 °C for 24h. The reaction was quenched at 0 °C with aqueous 0.1N HCl (750 ml) (pH = 5), and extracted with CH_2Cl_2 (2 x 400 ml). The organic phase was dried (sodium sulphate) and concentrated *in vacuo*. The residue was purified by flash column chromatography (SiO_2, gradient hexane:ethyl acetate 4:1 to hexane:ethyl acetate 3:2) to give **15** (17.6 g, 83 %) as a yellow solid.

Rf: 0.38 (hexane:ethyl acetate 3:7).

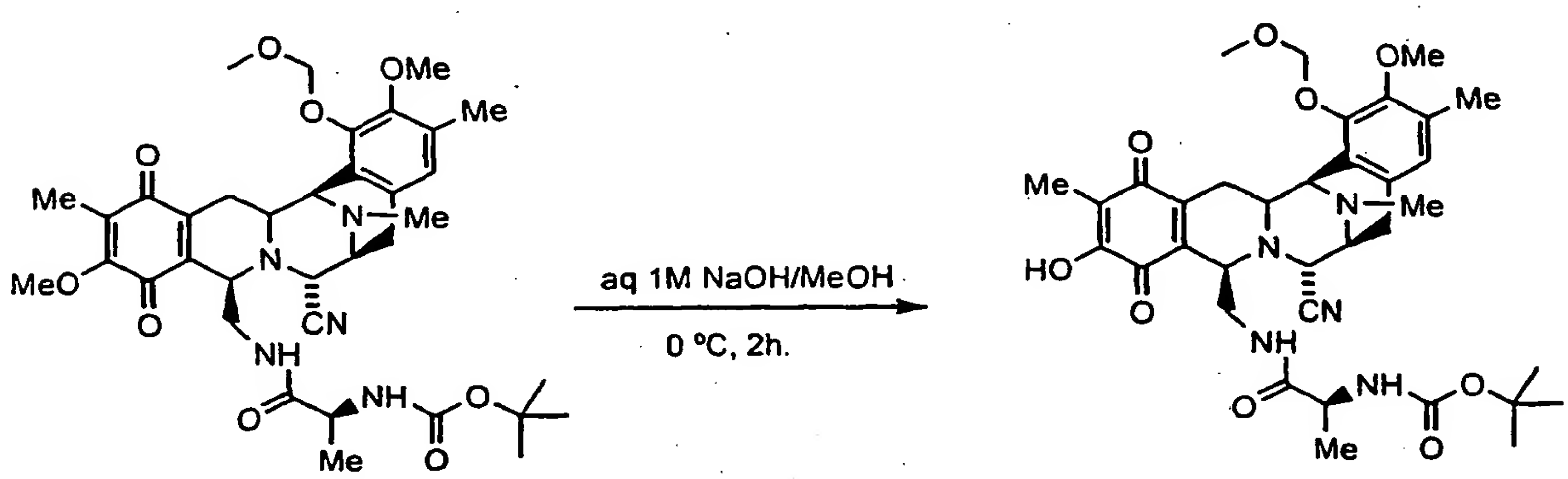
^1H NMR (300 MHz, $CDCl_3$): δ 6.73 (s, 1H), 5.35 (bs, 1H), 5.13 (s, 2H), 4.50 (bs, 1H), 4.25 (d, J= 2.7 Hz, 1H), 4.03 (d, J= 2.7 Hz, 1H), 3.97 (s, 3H), 3.84 (bs, 1H), 3.82-3.65 (m, 1H), 3.69 (s, 3H), 3.56 (s, 3H), 3.39-

3.37 (m, 1H), 3.20-3.00 (m, 5H), 2.46 (d, J= 18 Hz, 1H), 2.33 (s, 3H), 2.23 (s, 3H), 1.85 (s, 3H), 1.73-1.63 (m, 1H), 1.29 (s, 9H), 0.93 (d, J= 5.1 Hz, 3H)

^{13}C NMR (75 MHz, $CDCl_3$): δ 185.4, 180.9, 172.4, 155.9, 154.5, 149.0, 148.4, 141.6, 135.1, 131.0, 129.9, 127.6, 124.4, 123.7, 117.3, 99.1, 79.3, 60.7, 59.7, 58.4, 57.5, 56.2, 55.9, 55.0, 54.2, 50.0, 41.5, 39.9, 28.0, 25.2, 24.0, 18.1, 15.6, 8.5.

ESI-MS m/z: Calcd. for $C_{36}H_{47}N_5O_9$: 693.8. Found $(M+H)^+$: 694.3.

Example 3



15 16

To a flask containing **15** (8 g, 1.5 ml) in methanol (1.6 l) an aqueous solution of 1M sodium hydroxide (3.2 l) was added at 0 °C. The reaction was stirred for 2h at this temperature and then, quenched with 6M HCl to pH = 5. The mixture was extracted with ethyl acetate (3 x 1 l) and the combined organic layers were dried over sodium sulphate and concentrated *in vacuo*. The residue was purified by flash column chromatography (SiO_2, gradient $CHCl_3$ to $CHCl_3$:ethyl acetate 2:1) to afford **16** (5.3 mg, 68 %).

Rf: 0.48 (CH_3CN:H_2O 7:3, RP-C18)

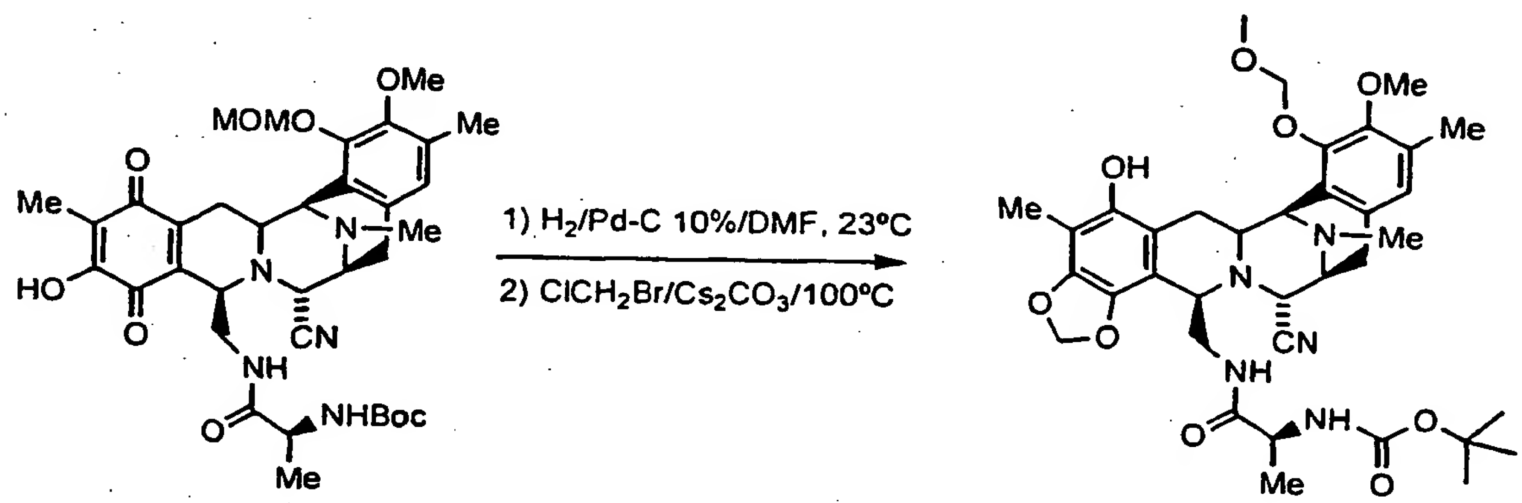
^1H NMR (300 MHz, $CDCl_3$): δ 6.73 (s, 1H), 5.43 (bs, 1H), 5.16 (s, 2H), 4.54 (bs, 1H), 4.26 (d, J= 1.8 Hz, 1H), 4.04 (d, J= 2.7 Hz 1H), 3.84 (bs,

1H), 3.80-3.64 (m, 1H), 3.58 (s, 3H), 3.41-3.39 (m, 1H), 3.22-3.06 (m, 5H), 2.49 (d, J= 18.6 Hz 1H), 2.35 (s, 3H), 2.30-2.25 (m, 1H), 2.24 (s, 3H), 1.87 (s, 3H), 1.45-1.33 (m, 1H), 1.19 (s, 9H), 1.00 (br d, J= 6.6 Hz 3H)

^{13}C NMR (75 MHz,□CDCl$_3$): δ 184.9, 180.9, 172.6, 154.7, 151.3, 149.1, 148.6, 144.7, 132.9, 131.3, 129.8, 124.5, 123.7, 117.3, 116.8, 99.1, 79.4, 59.8, 58.6, 57.7, 56.2, 55.6, 54.9, 54.5, 50.1, 41.6, 40.1, 28.0, 25.3, 24.4, 18.1, 15.7, 8.0.

ESI-MS m/z: Calcd. for $C_{35}H_{45}N_5O_9$: 679.7. Found $(M+H)^+$: 680.3.

Example 4



To a degassed solution of compound **16** (1.8 g, 2.64 mmol) in DMF (221 ml) 10 % Pd/C (360 mg) was added and stirred under H_2 (atmospheric pressure) for 45 min. The reaction was filtered through celite under argon, to a flask containing anhydrous Cs_2CO_3 (2.58 g, 7.92 mmol). Then, bromochloromethane (3.40 ml 52.8 mmol), was added and the tube was sealed and stirred at 100 °C for 2h. The reaction was cooled, filtered through a pad of celite and washed with CH_2Cl_2. The organic layer was concentrated and dried (sodium sulphate) to afford **17** as a brown oil that was used in the next step with no further purification.

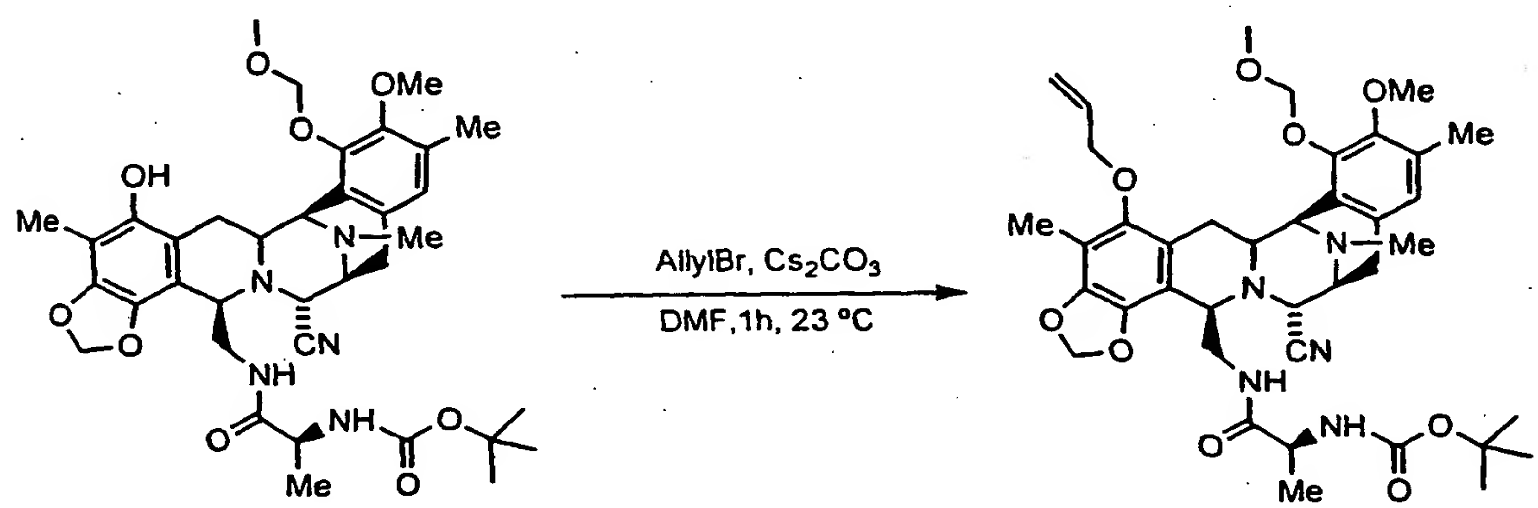
Rf: 0.36 (hexane:ethyl acetate 1:5, SiO_2).

^1H NMR (300 MHz, $CDCl_3$): δ 6.68 (s, 1H), 6.05 (bs, 1H), 5.90 (s, 1H), 5.79 (s, 1H), 5.40 (bs, 1H), 5.31-5.24 (m, 2H), 4.67 (d, J= 8.1 Hz, 1H), 4.19 (d, J= 2.7 Hz, 1H), 4.07 (bs, 1H), 4.01 (bs, 1H), 3.70 (s, 3H), 3.67 (s, 3H), 3.64-2.96 (m, 5H), 2.65 (d, J=18.3 Hz, 1H), 2.33 (s, 3H), 2.21 (s, 3H), 2.04 (s, 3H), 2.01-1.95 (m, 1H), 1.28 (s, 9H), 0.87 (d, J= 6.3 Hz, 3H)

^{13}C NMR (75 MHz, $CDCl_3$): δ 172.1, 162.6, 154.9, 149.1, 145.7, 135.9, 130.8, 130.7, 125.1, 123.1, 117.8, 100.8, 99.8, 76.6, 59.8, 59.2, 57.7, 57.0, 56.7, 55.8, 55.2, 49.5, 41.6, 40.1, 36.5, 31.9, 31.6, 29.7, 28.2, 26.3, 25.0, 22.6, 18.2, 15.8, 14.1, 8.8.

ESI-MS m/z: Calcd. for $C_{36}H_{47}N_5O_9$: 693.34. Found $(M+H)^+$: 694.3.

Example 5



17 18

To a flask containing a solution of **17** (1.83 g, 2.65 mmol) in DMF (13 ml), Cs_2CO_3 (2.6 g, 7.97 mmol), and allyl bromide (1.15 ml, 13.28 mmol) were added at 0° C. The resulting mixture was stirred at 23 °C for 1h. The reaction was filtered through a pad of celite and washed with CH_2Cl_2. The organic layer was dried and concentrated (sodium sulphate). The residue was purified by flash column chromatography (SiO_2, $CHCl_3$:ethyl acetate 1:4) to afford **18** (1.08 mg, 56 %) as a white solid.

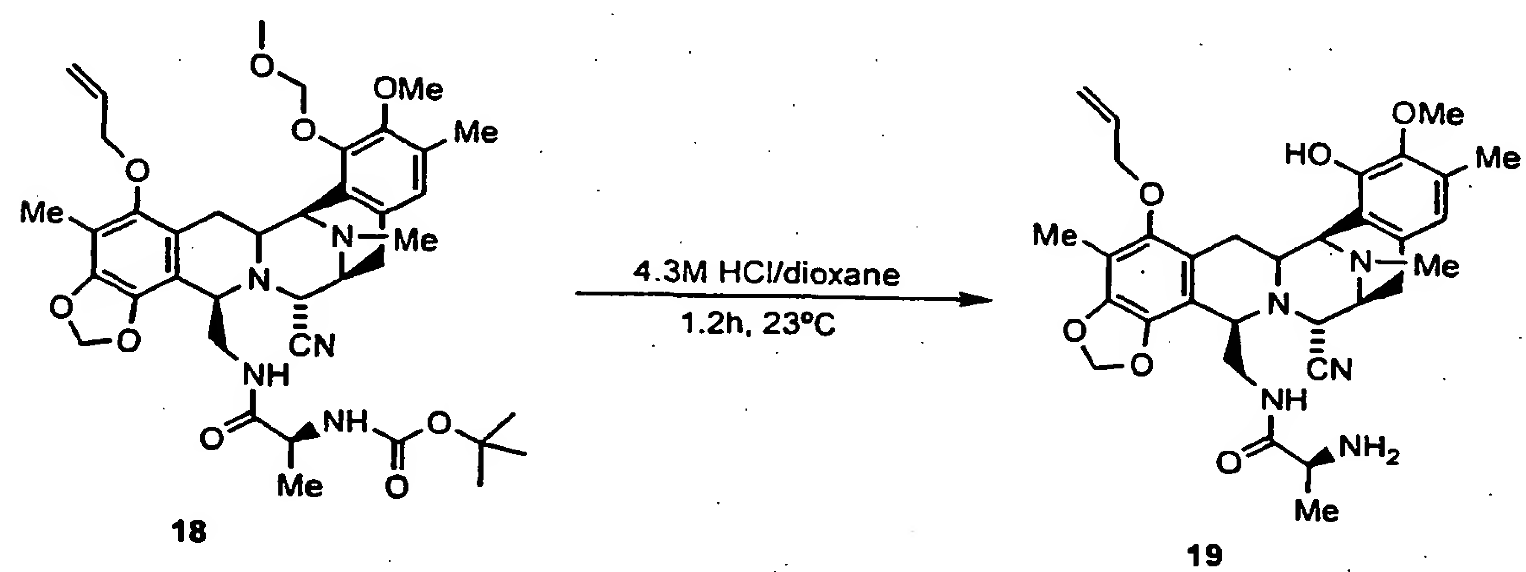
Rf: 0.36 ($CHCl_3$:ethyl acetate 1:3).

^1H NMR (300 MHz, $CDCl_3$): δ 6.70 (s, 1H), 6.27-6.02 (m, 1H), 5.94 (s, 1H), 5.83 (s, 1H), 5.37 (dd, J_1= 1.01 Hz, J_2= 16.8 Hz, 1H), 5.40 (bs, 1H), 5.25 (dd, J_1= 1.0 Hz, J_2= 10.5 Hz, 1H), 5.10 (s, 2H), 4.91 (bs, 1H), 4.25-4.22 (m, 1H), 4.21 (d, J= 2.4 Hz, 1H), 4.14-4.10 (m, 1H), 4.08 (d, J=2.4 Hz, 1H), 4.00 (bs, 1H), 3.70 (s, 3H), 3.59 (s, 3H), 3.56-3.35 (m, 2H), 3.26-3.20 (m, 2H), 3.05-2.96 (dd, J_1= 8.1 Hz, J_2=18 Hz, 1H), 2.63 (d, J=18 Hz, 1H), 2.30 (s, 3H), 2.21 (s, 3H), 2.09 (s, 3H), 1.91-1.80 (m, 1H), 1.24 (s, 9H), 0.94 (d, J= 6.6 Hz, 3H)

^{13}C NMR (75 MHz, $CDCl_3$): δ 172.0, 154.8, 148.8, 148.6, 148.4, 144.4, 138.8, 133.7, 130.9, 130.3, 125.1, 124.0, 120.9, 117.8, 117.4, 112.8, 112.6, 101.1, 99.2, 73.9, 59.7, 59.3, 57.7, 56.9, 56.8, 56.2, 55.2, 40.1, 34.6, 31.5, 28.1, 26.4, 25.1, 22.6, 18.5, 15.7, 14.0, 9.2.

ESI-MS m/z: Calcd. for $C_{39}H_{51}N_5O_9$: 733.4. Found $(M+H)^+$: 734.4.

Example 6



To a solution of **18** (0.1 g, 0.137 mmol) in dioxane (2 ml), 4.2M HCl/dioxane (1.46 ml) was added and the mixture was stirred for 1.2h at 23 °C. The reaction was quenched at 0 °C with sat. Aqueous sodium bicarbonate (60 ml) and extracted with ethyl acetate (2x70 ml). The organic layers were dried (sodium sulphate) and concentrated *in vacuo* to afford **19** (267 mg, 95 %) as a white solid that was used in subsequent reactions with no further purification.

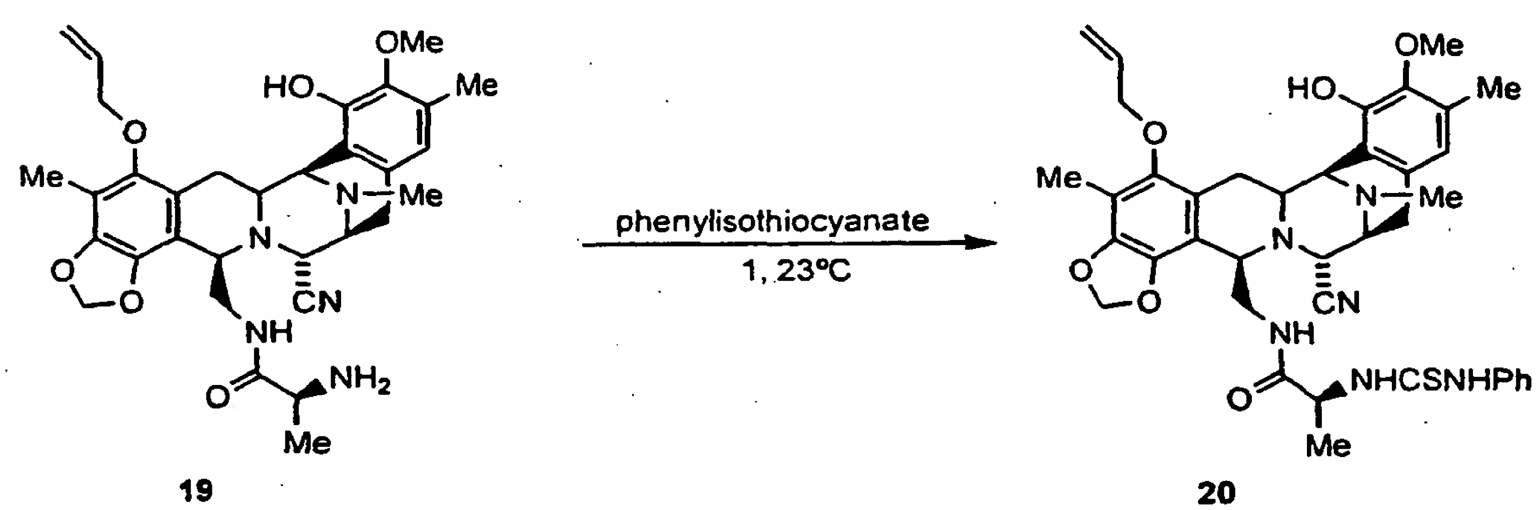
Rf: 0.17 (ethyl acetate:methanol 10:1, SiO_2)

^{1}H NMR (300 MHz, $CDCl_3$): δ 6.49 (s, 1H), 6.12-6.00 (m, 1H), 5.94 (s, 1H), 5.86 (s, 1H), 5.34 (dd, *J*= 1.0 Hz, *J*= 17.4 Hz, 1H), 5.25 (dd, *J*= 1.0 Hz, *J*= 10.2 Hz, 1H), 4.18-3.76 (m, 5H), 3.74 (s, 3H), 3.71-3.59 (m, 1H), 3.36-3.20 (m, 4H), 3.01-2.90 (m, 1H), 2.60 (d, *J*= 18.0 Hz, 1H), 2.29 (s, 3H), 2.24 (s, 3H), 2.11 (s, 3H), 1.97-1.86 (m, 1H), 0.93 (d, *J*= 8.7 Hz, 3H)

^{13}C NMR (75 MHz, $CDCl_3$): δ 175.5, 148.4, 146.7, 144.4, 142.4, 138.9, 133.7, 131.3, 128.3, 120.8, 117.9, 117.4, 113.8, 112.4, 101.1, 74.2, 60.5, 59.1, 56.5, 56.1, 56.3, 56.0, 55.0, 50.5, 41.6, 39.5, 29.5, 26.4, 24.9, 21.1, 15.5, 9.33.

ESI-MS m/z: Calcd. for $C_{32}H_{39}N_5O_6$: 589. Found $(M+H)^+$: 590.

Example 7



To a solution of **19** (250 mg, 0.42 mmol) in CH_2Cl_2 (1.5 mmol), phenyl isothiocyanate (0.3 ml, 2.51 mmol) was added and the mixture was stirred at 23° C for 1h. The reaction was concentrated *in vacuo* and the residue was purified by flash column chromatography (SiO_2, gradient Hexane to 5:1 hexane:ethyl acetate) to afford **20** (270 mg, 87 %) as a white solid.

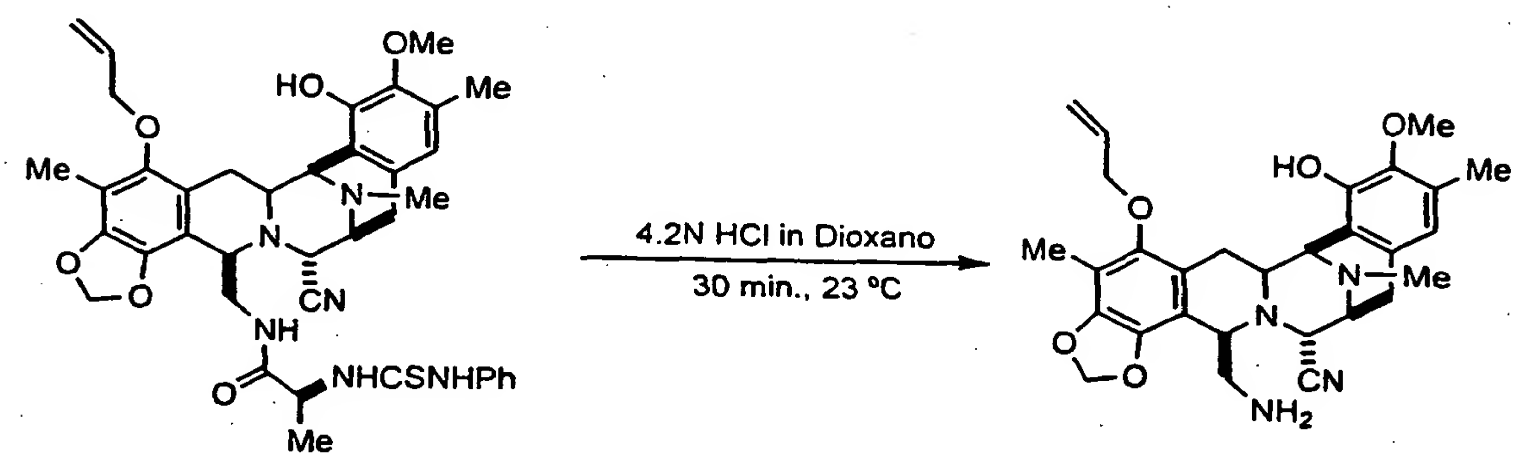
Rf: 0.56 ($CHCl_3$:ethyl acetate 1:4).

^1H NMR (300 MHz, $CDCl_3$): δ 8.00 (bs, 1H), 7.45-6.97 (m, 4H), 6.10 (s, 1H), 6.08-6.00 (m, 1H), 5.92 (s, 1H), 5.89 (s, 1H), 5.82 (s, 1H), 5.40 (dd, J= 1.5 Hz, J= 17.1 Hz, 1H), 3.38 (bs, 1H), 5.23 (dd, J= 1.5 Hz, J= 10.5 Hz, 1H), 4.42-4.36 (m, 1H), 4.19-4.03 (m, 5H), 3.71 (s, 3H), 3.68-3.17 (m, 4H), 2.90 (dd, J=7.8 Hz, J= 18.3 Hz, 1H), 2.57 (d, J= 18.3 Hz, 1H), 2.25 (s, 3H), 2.12 (s, 3H), 2.10 (s, 3H), 1.90 (dd, J= 12.3 Hz, J= 16.5 Hz, 1H), 0.81 (d, J= 6.9 Hz, 3H).

^{13}C NMR (75 MHz, $CDCl_3$): δ 178.4, 171.6, 148.6, 146.8, 144.3, 142.7, 138.7, 136.2, 133.6, 130.7, 129.8, 126.6, 124.2, 124.1, 120.9, 120.5, 117.7, 117.4, 116.7, 112.6, 112.5, 101.0, 74.0, 60.6, 59.0, 57.0, 56.2, 56.1, 55.0, 53.3, 41.4, 39.7, 26.3, 24.8, 18.3, 15.5, 9.2.

ESI-MS m/z: Calcd. for $C_{39}H_{44}N_6O_6S$: 724.8 Found $(M+H)^+$: 725.3.

Example 8



20 21

To a solution of **20** (270 mg, 0.37 mmol) in dioxane (1 ml), 4.2N HCl/dioxane (3.5 ml) was added and the reaction was stirred at 23 °C for 30 min. Then, ethyl acetate (20 ml) and H_2O (20 ml) were added and the organic layer was decanted. The aqueous phase was basified with saturated aqueous sodium bicarbonate (60 ml) (pH = 8) at 0 °C and then, extracted with CH_2Cl_2 (2 x 50 ml). The combined organic extracts were dried (sodium sulphate), and concentrated *in vacuo*. The residue was purified by flash column chromatography (SiO_2, ethyl

acetate:methanol 5:1) to afford compound **21** (158 mg, 82%) as a white solid.

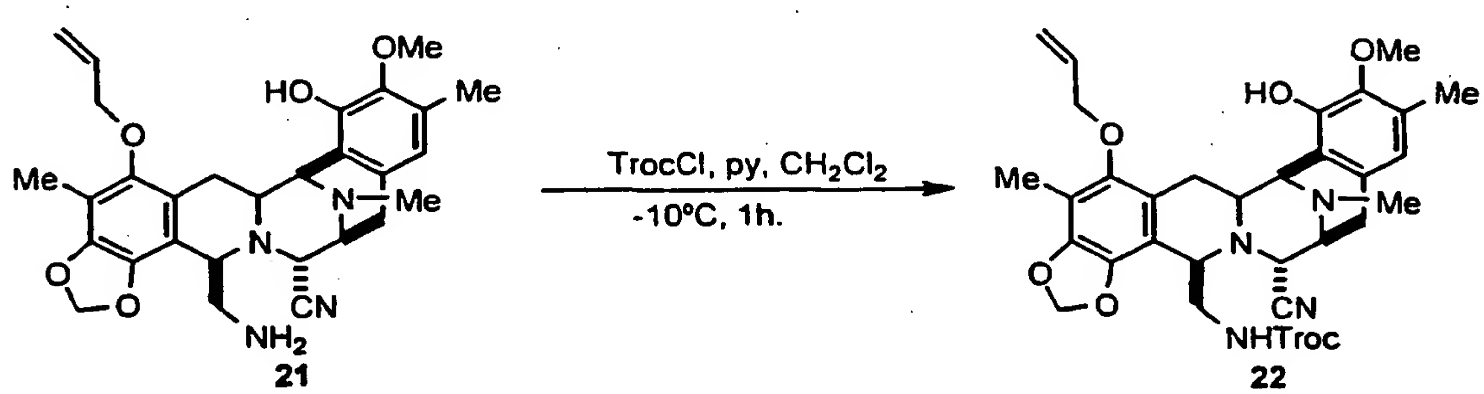
Rf: 0.3 (ethyl acetate:methanol 1:1).

^1H NMR (300 MHz, $CDCl_3$): δ 6.45 (s, 1H), 6.12-6.03 (m, 1H), 5.91 (s, 1H), 5.85 (s, 1H), 5.38 (dd, J_1= 1.2 Hz, J_2= 17.1 Hz, 1H), 5.24 (dd, J_1= 1.2 Hz, J_2= 10.5 Hz, 1H), 4.23-4.09 (m, 4H), 3.98 (d, J= 2.1 Hz, 1H), 3.90 (bs, 1H), 3.72 (s, 3H), 3.36-3.02 (m, 5H), 2.72-2.71 (m, 2H), 2.48 (d, J= 18.0 Hz, 1H), 2.33 (s, 3H), 2.22 (s, 3H), 2.11 (s, 3H), 1.85 (dd, J_1= 11.7 Hz, J_2= 15.6 Hz, 1H)).

^{13}C NMR (75 MHz, $CDCl_3$): δ 148.4, 146.7, 144.4, 142.8, 138.8, 133.8, 130.5, 128.8, 121.5, 120.8, 118.0, 117.5, 116.9, 113.6, 112.2, 101.1, 74.3, 60.7, 59.9, 58.8, 56.6, 56.5, 55.3, 44.2, 41.8, 29.7, 26.5, 25.7, 15.7, 9.4.

ESI-MS m/z: Calcd. for $C_{29}H_{34}N_4O_5$: 518.3. Found $(M+H)^+$: 519.2.

Example 9



To a solution of **21** (0.64 g, 1.22 mmol) in CH_2Cl_2 (6.13 ml), pyridine (0.104 ml, 1.28 mmol) and 2,2,2-trichloroethyl chloroformate (0.177 ml, 1.28 mmol) were added at -10 °C. The mixture was stirred at this temperature for 1h and then, the reaction was quenched by addition of 0.1N HCl (10 ml) and extracted with CH_2Cl_2 (2 x 10 ml). The organic layer was dried over sodium sulphate and concentrated *in vacuo*. The residue was purified by flash column chromatography

(SiO_2; (hexane:ethyl acetate 1:2) to afford **22** (0.84 g, 98%) as a white foam solid.

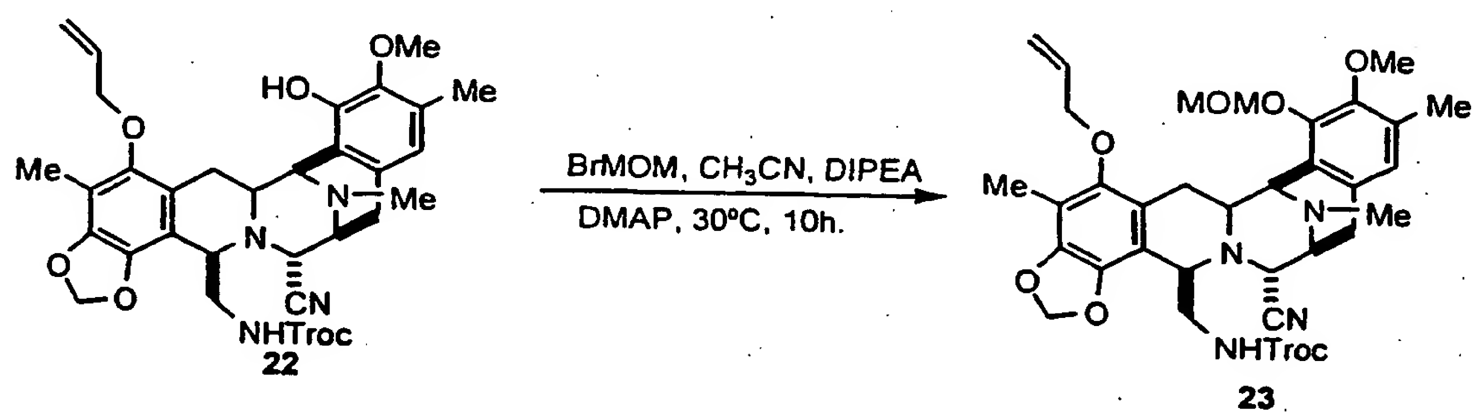
Rf: 0.57 (ethyl acetate:methanol 5:1).

^{1}H NMR (300 MHz, $CDCl_3$): δ 6.50 (s, 1H), 6.10-6.00 (m, 1H), 6.94 (d, *J*= 1.5 Hz, 1H), 5.87 (d, *J*= 1.5 Hz, 1H), 5.73 (bs, 1H), 5.37 (dq, J_1= 1.5 Hz, J_2= 17.1 Hz, 1H), 5.26 (dq, J_1= 1.8 Hz, J_2= 10.2 Hz, 1H), 4.60 (d, *J*= 12 Hz, 1H), 4.22-4.10 (m, 4H), 4.19 (d, *J*= 12 Hz, 1H), 4.02 (m, 2H), 3.75 (s, 3H), 3.37-3.18 (m, 5H), 3.04 (dd, J_1= 8.1 Hz, J_2= 18 Hz, 1H), 2.63 (d, *J*= 18 Hz, 1H), 2.31 (s, 3H), 2.26 (s, 3H), 2.11 (s, 3H), 1.85 (dd, J_1= 12.3 Hz, J_2= 15.9 Hz, 1H).

^{13}C NMR (75 MHz, $CDCl_3$) δ 154.3, 148.5, 146.7, 144.5, 142.8, 139.0, 133.8, 130.7, 128.7, 121.3, 120.8, 117.8, 117.7, 116.8, 112.7, 101.2, 77.2, 74.3, 60.7, 59.9, 57.0, 56.4, 55.3, 43.3, 41.7, 31.6, 26.4, 25.3, 22.6, 15.9, 14.1, 9.4.

ESI-MS m/z: Calcd. for $C_{32}H_{35}Cl_3N_4O_7$: 694.17. Found $(M+H)^+$: 695.2.

Example 10

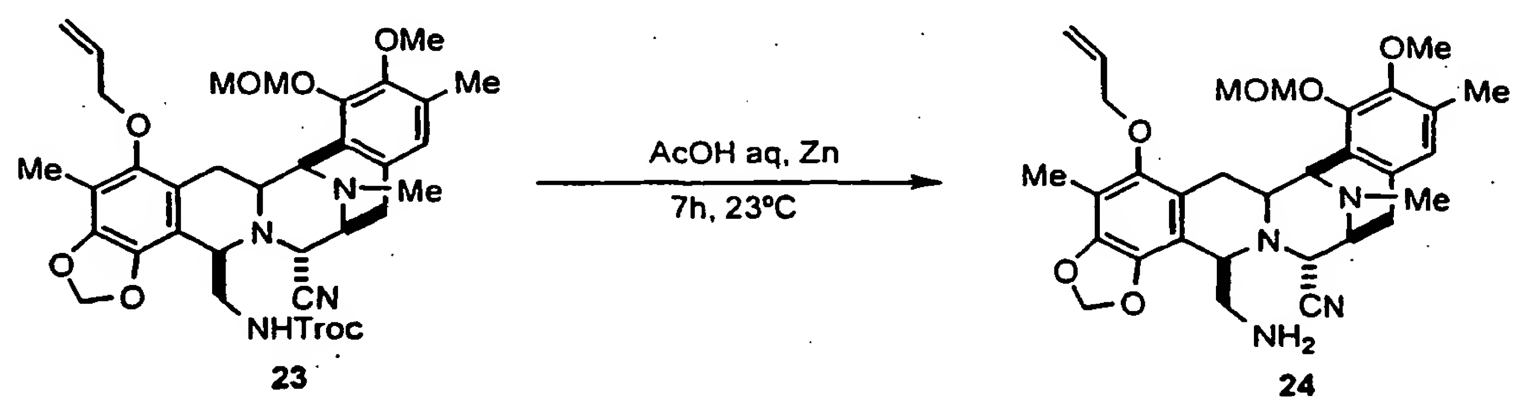


To a solution of **22** (0.32 g, 0.46 mmol) in CH_3CN (2.33 ml), diisopropylethylamine (1.62 ml, 9.34 mmol), bromomethyl methyl ether (0.57 ml, 7.0 mmol) and dimethylaminopyridine (6 mg, 0.046 mmol) were added at 0 °C. The mixture was heated at 30 °C for 10h. Then, the reaction was diluted with dichloromethane (30 ml) and poured in an aqueous solution of HCl at pH = 5 (10 ml). The organic layer was dried over sodium sulphate and the solvent was eliminated under reduced

pressure to give a residue which was purified by flash column chromatography (SiO_2, hexane:ethyl acetate 2:1) to afford **23** (0.304 g, 88%) as a white foam solid.

Rf: 0.62 (hexane:ethyl acetate 1:3).
^{1}H NMR (300 MHz, $CDCl_3$): δ 6.73 (s, 1H), 6.10 (m, 1H), 5.94 (d, J= 1.5 Hz, 1H), 5.88 (d, J= 1.5 Hz, 1H), 5.39 (dq, J_1= 1.5 Hz, J_2= 17.1 Hz, 1H), 5.26 (dq, J_1= 1.8 Hz, J_2= 10.2 Hz, 1H), 5.12 (s, 2H), 4.61 (d, J= 12 Hz, 1H), 4.55 (t, J= 6.6 Hz, 1H), 4.25 (d, J= 12 Hz, 1H), 4.22-4.11 (m, 4H), 4.03 (m, 2H), 3.72 (s, 3H), 3.58 (s, 3H), 3.38-3.21 (m, 5H), 3.05 (dd, J_1= 8.1 Hz, J_2= 18 Hz, 1H), 2.65 (d, J= 18 Hz, 1H), 2.32 (s, 3H), 2.23 (s, 3H), 2.12 (s, 3H), 1.79 (dd, J_1= 12.3 Hz, J_2= 15.9 Hz, 1H);
^{13}C NMR (75 MHz, $CDCl_3$) δ 154.3, 148.6, 148.4, 144.5, 139.0, 133.6, 130.6, 130.1, 125.07, 124.7, 124.0, 121.1, 117.7, 112.6, 101.2, 99.2, 77.2, 74.4, 74.1, 59.8, 59.8, 57.7, 57.0, 56.8, 56.68, 55.3, 43.2, 41.5, 26.4, 25.2, 15.9, 9.3.
ESI-MS m/z: Calcd. for $C_{34}H_{39}Cl_3N_4O_8$: 738.20. Found $(M+H)^+$: 739.0.

Example 11



To a suspension of **23** (0.304 g, 0.41 mmol) in 90% aqueous acetic acid (4 ml), powder zinc (0.2 g, 6.17 mmol) was added and the reaction was stirred for 7 hour at 23 °C. The mixture was filtered through a pad of celite which was washed with CH_2Cl_2. The organic layer was washed with an aqueous sat. solution of sodium bicarbonate

(pH = 9) (15 ml) and dried over sodium sulphate. The solvent was eliminated under reduced pressure to give **24** (0.191 g, 83%) as a white solid.

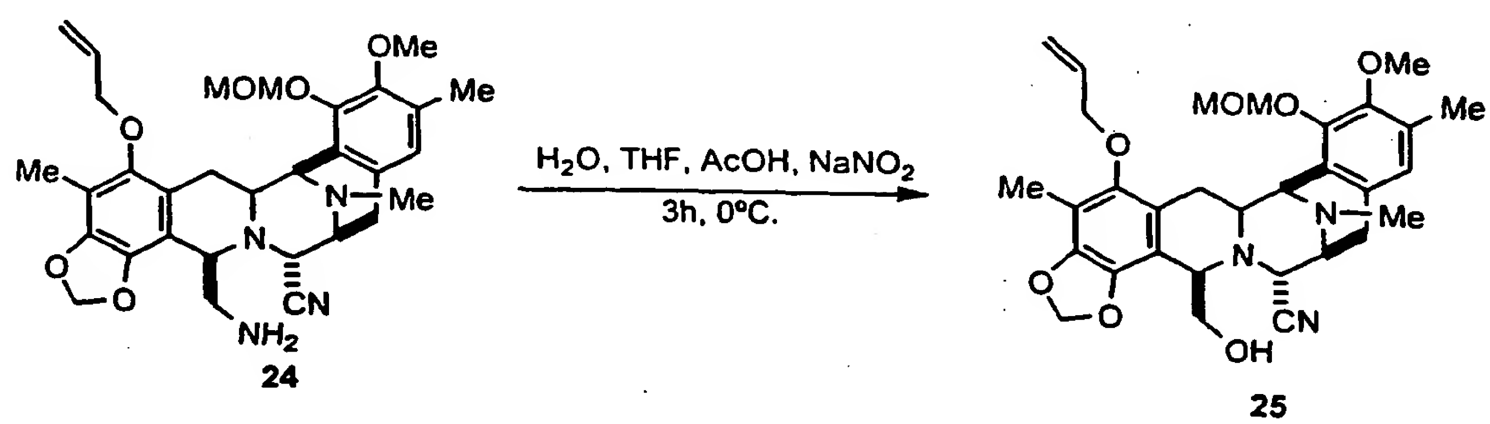
Rf: 0.3 (ethyl acetate:methanol 5:1).

^1H NMR (300 MHz, $CDCl_3$): δ 6.68 (s, 1H), 6.09 (m, 1H), 5.90 (d, J= 1.5 Hz, 1H), 5.83 (d, J= 1.5 Hz, 1H), 5.39 (dq, J_1= 1.5 Hz, J_2= 17.1 Hz, 1H), 5.25 (dq, J_1= 1.5 Hz, J_2= 10.2 Hz, 1H), 5.10 (s, 2H), 4.22-4.09 (m, 3H), 3.98 (d, J= 2.4 Hz, 1H), 3.89 (m, 1H), 3.69 (s, 3H), 3.57 (s, 3H), 3.37-3.17 (m, 3H), 3.07 (dd, J_1= 8.1 Hz, J_2= 18 Hz, 1H), 2.71 (m, 2H), 2.48 (d, J= 18 Hz, 1H), 2.33 (s, 3H), 2.19 (s, 3H), 2.17 (s, 3H), 1.80 (dd, J_1= 12.3 Hz, J_2= 15.9 Hz, 1H)

^{13}C NMR (75 MHz, $CDCl_3$): δ 148.5, 148.2, 144.3, 138.7, 133.7, 130.7, 129.9, 125.0, 123.9, 121.3, 117.9, 117.5, 113.6, 112.0, 101.0, 99.2, 74.0, 59.8, 59.7, 58.8, 57.6, 57.0, 56.2, 55.2, 44.2, 41.5, 31.5, 26.4, 25.6, 22.5, 16.7, 14.0, 9.2.

ESI-MS m/z: Calcd. for $C_{31}H_{38}N_4O_6$: 562.66. Found $(M+H)^+$: 563.1.

Example 12



To a solution of **24** (20 mg, 0.035 mmol), in H_2O (0.7 mmol) and THF (0.7 mmol), $NaNO_2$ (12 mg, 0.17 mmol) and 90% aqueous AcOH (0.06 ml) were added at 0 °C and the mixture was stirred at 0 °C for 3h. After dilution with CH_2Cl_2 (5 ml), the organic layer was washed with water (1 ml), dried over sodium sulphate and concentrated *in vacuo*.

The residue was purified by flash column chromatography (SiO_2, hexane:ethyl acetate 2:1) to afford **25** (9.8 mg, 50%) as a white solid.

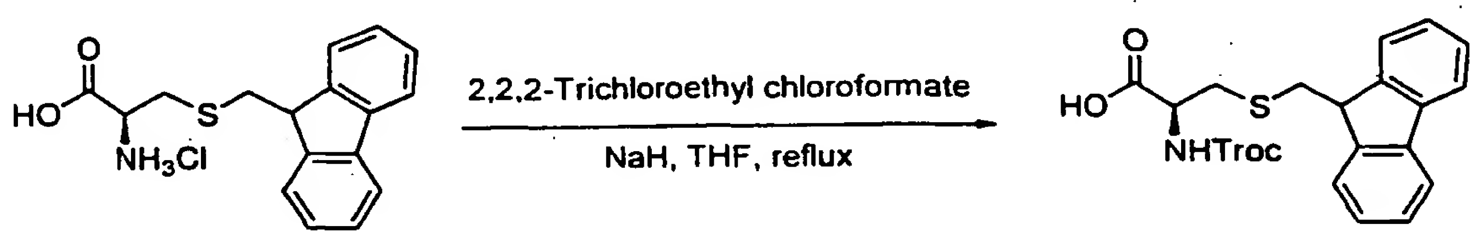
Rf: 0.34 (hexane:ethyl acetate 1:1).

^{1}H NMR (300 MHz, $CDCl_3$): δ 6.71 (s, 1H), 6.11 (m, 1H), 5.92 (d, J= 1.5 Hz, 1H), 5.87 (d, J= 1.5 Hz, 1H), 5.42 (dq, J_1= 1.5 Hz, J_2= 17.1 Hz, 1H), 5.28 (dq, J_1= 1.5 Hz, J_2= 10.2 Hz, 1H), 5.12 (s, 2H), 4.26-4.09 (m, 3H), 4.05 (d, J= 2.4 Hz, 1H), 3.97 (t, J= 3.0 Hz, 1H), 3.70 (s, 3H), 3.67-3.32 (m, 4H), 3.58 (s, 3H), 3.24 (dd, J_1= 2.7 Hz, J_2= 15.9 Hz, 1H), 3.12 (dd, J_1= 8.1 Hz, J_2= 18.0 Hz, 1H), 2.51 (d, J= 18 Hz, 1H), 2.36 (s, 3H), 2.21 (s, 3H), 2.12 (s, 3H), 1.83 (dd, J_1= 12.3 Hz, J_2= 15.9 Hz, 1H)

^{13}C NMR (75 MHz, $CDCl_3$) δ 148.7, 148.4, 138.9, 133.7, 131.1, 129.4, 125.1, 123.9, 120.7, 117.6, 117.5, 113.2, 112.3, 101.1, 99.2, 74.0, 63.2, 59.8, 59.7, 57.9, 57.7, 57.0, 56.5, 55.2, 41.6, 29.6, 26.1, 25.6, 22.6, 15.7, 9.2.

ESI-MS m/z: Calcd. for $C_{31}H_{37}N_3O_7$: 563.64. Found $(M+H)^+$: 564.1.

Example 13



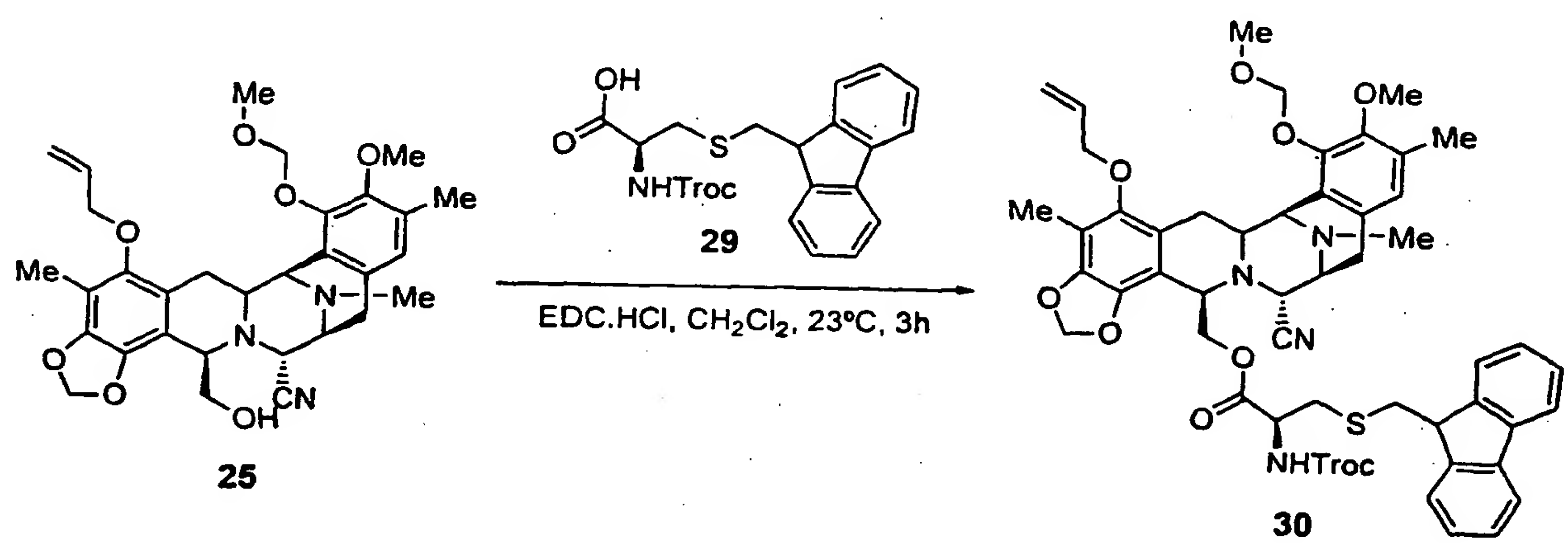
29

The starting material (2.0 g, 5.90 mmol) was added to a suspension of sodium hydride (354 mg, 8.86 mmol) in THF (40 ml) at 23 °C, following the suspension was treated with allyl chloroformate (1.135 ml, 8.25 mmol) at 23 °C and then refluxed for 3 hours. The suspension was cooled, filtered off, the solid washed with ethyl acetate (100 ml), and the filtrate was concentrated. The oil crude was ground with hexane (100 ml) and kept at 4°C overnight. After, the solvent was decanted and the light yellow slurry was treated with CH_2Cl_2 (20 ml),

and precipitated with hexane (100 ml). After 10 minutes, the solvent was decanted again. The operation was repeated until appearing a white solid. The white solid was filtered off and dried to afford compound **29** (1.80 g, 65%) as a white solid.

^{1}H-NMR (300 MHz, $CDCl_3$): δ 7.74 (d, J= 7.5 Hz, 2H), 7.62 (d, J= 6.9 Hz, 2H), 7.33 (t, J= 7.5 Hz, 2H), 7.30 (t, J= 6.3 Hz, 2H), 5.71 (d, J= 7.8 Hz, 1H), 4.73 (d, J= 7.8 Hz, 2H), 4.59 (m, 1H), 4.11 (t, J= 6.0 Hz, 1H), 3.17 (dd, J= 6.0 Hz, J= 2.7 Hz, 2H), 3.20 (dd, J= 5.4 Hz, J= 2.1 Hz, 2H).
^{13}C-NMR (75 MHz, $CDCl_3$): δ 173.6, 152.7, 144.0, 139.7, 137.8, 126.0, 125.6, 123.4, 118.3, 73.4, 52.4, 45.5, 35.8, 33.7.
ESI-MS m/z: Calcd.. for $C_{20}H_{18}Cl_3NO_4S$: 474.8. Found $(M+Na)^+$: 497.8

Example 14

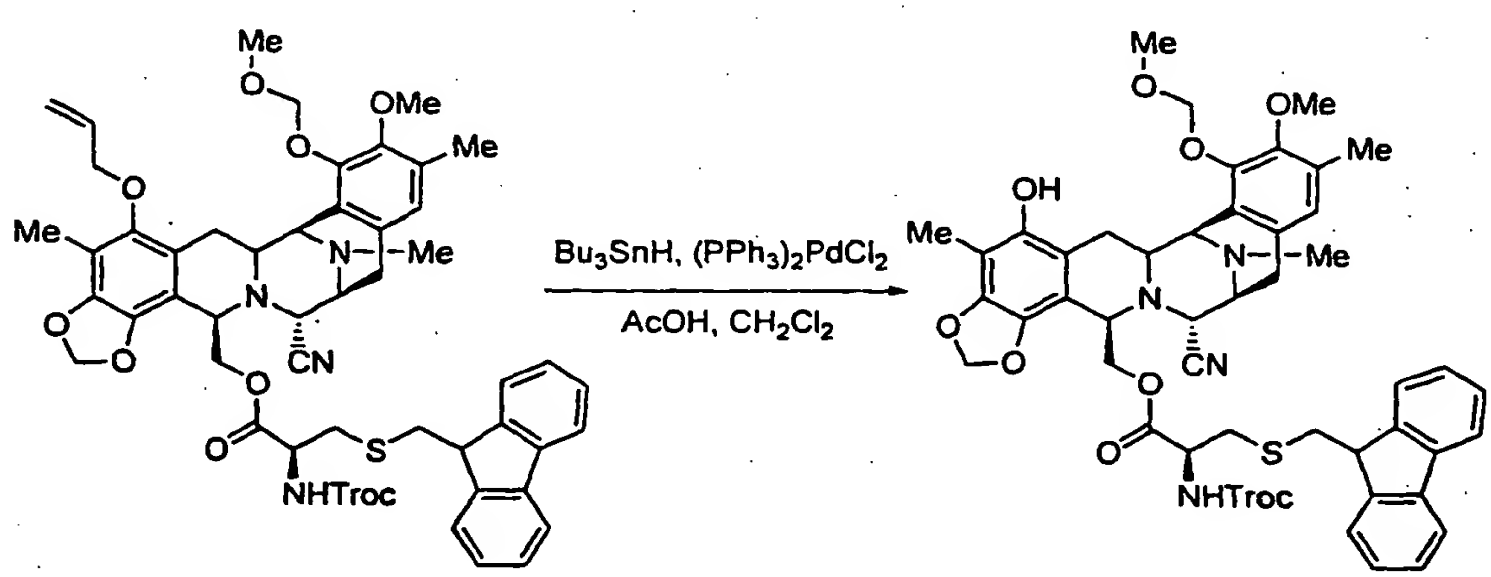


A mixture of compound **25** (585 mg, 1.03 mmol) and compound **29** (1.47 mg, 3.11 mmol) were azeotroped with anhydrous toluene (3 x 10 ml). To a solution of **25** and **29** in anhydrous CH_2Cl_2 (40 ml) was added DMAP (633 mg, 5.18 mmol) and EDC·HCl (994 mg, 5.18 mmol) at 23 °C. The reaction mixture was stirred at 23 °C for 3 hours. The mixture was partitioned with saturated aqueous solution of sodium bicarbonate (50 ml) and the layers were separated. The aqueous layer was washed with CH_2Cl_2 (50 ml). The combined organic layers were

dried over sodium sulphate, filtered and concentrated. The crude was purified by flash column chromatography (ethyl acetate/hexane 1:3) to obtain **30** (1.00 g, 95%) as a pale cream yellow solid.

^{1}H-NMR (300 MHz, $CDCl_3$): δ 7.72 (m, 2H), 7.52 (m, 2H), 7.38 (m, 2H), 7.28 (m, 2H), 6.65 (s, 1H), 6.03 (m, 1H), 5.92 (d, *J*= 1.5 Hz, 1H), 5.79 (d, *J*= 1.5 Hz, 1H), 5.39 (m, 1H), 5.29 (dq, *J*= 10.3 Hz, *J*= 1.5 Hz, 1H), 5.10 (s, 2H), 4.73 (d, *J*= 11.9 Hz, 1H), 4.66 (d, *J*= 11.9 Hz, 1H), 4.53 (m, 1H), 4.36-3.96 (m, 9H), 3.89 (t, *J*= 6.4 Hz, 1H), 3.71 (s, 3H), 3.55 (s, 3H), 3.33 (m, 1H), 3.20 (m, 2H), 2.94 (m, 3H), 2.59 (m, 1H), 2.29 (s, 3H), 2.23 (s, 3H), 2.02 (s, 3H), 1.83 (dd, *J*= 16.0 Hz, *J*= 11.9 Hz, 1H).
^{13}C-NMR (75 MHz, $CDCl_3$): δ 169.7, 154.0, 148.8, 148.4, 145.7, 144.5, 140.9, 139.0, 133.7, 130.9, 130.6, 127.6, 127.0, 124.8, 124.6, 124.1, 120.8, 119.9, 118.2, 117.7, 117.3, 112.7, 112.1, 101.3, 99.2, 74.7, 73.9, 64.4, 59.8, 57.7, 57.0, 56.8, 55.4, 53.3, 46.7, 41.4, 36.5, 34.7, 31.5, 26.4, 24.9, 22.6, 15.7, 14.0, 9.1.
ESI-MS m/z: Calcd.. for $C_{51}H_{53}Cl_3N_4O_{10}S$: 1020.4. Found $(M+H)^+$: 1021.2

Example 15



30 **31**

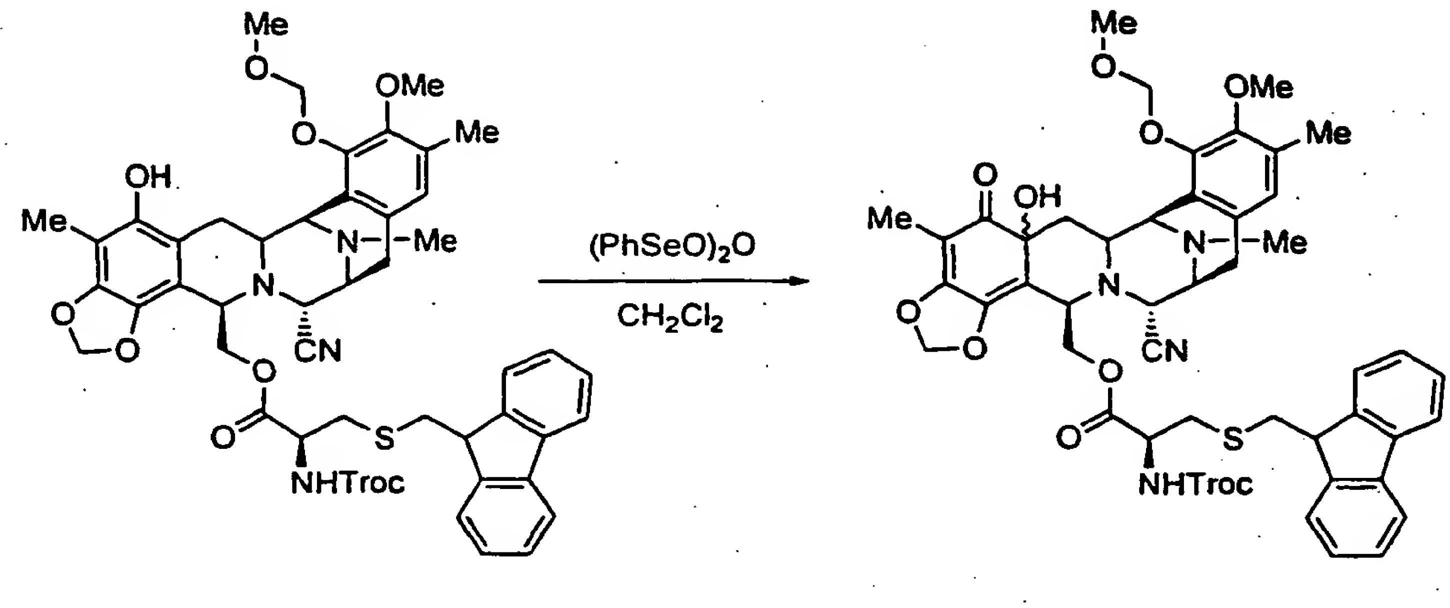
To a solution of **30** (845 mg, 0.82 mmol), acetic acid (500 mg, 8.28 mmol) and $(PPh_3)_2PdCl_2$ (29 mg, 0.04 mmol) in anhydrous CH_2Cl_2 20 ml at 23 °C was added, dropwise, Bu_3SnH (650 mg, 2.23 mmol). The reaction mixture was stirred at this temperature for 15 min., bubbling was. The crude was quenched with water (50ml) and extracted with CH_2Cl_2 (3 x 50 ml). The organic layers were dried over sodium sulphate, filtered and concentrated. The crude was purified by flash column chromatography (ethyl acetate/hexane in gradient from 1:5 to 1:3) to obtain compound **31** (730 mg, 90%) as a pale cream yellow solid.

^1H-NMR (300 MHz, $CDCl_3$): δ 7.72 (m, 2H), 7.56 (m, 2H), 7.37 (m, 2H), 7.30 (m, 2H), 6.65 (s, 1H), 5.89 (s, 1H), 5.77 (s, 1H), 5.74 (s, 1H), 5.36 (d, *J*= 5.9 Hz, 1H), 5.32 (d, *J*= 5.9 Hz, 1H), 5.20 (d, *J*= 9.0, 1H), 4.75 (d, *J*= 12.0 Hz, 1H), 4.73 (m, 1H), 4.48 (d, *J*= 11.9 Hz, 1H), 4.08 (m, 4H), 3.89 (m, 1H), 3.86, (t, *J*= 6.2 Hz, 1H), 3.70 (s, 3H), 3.69 (s, 3H), 3.38 (m, 1H), 3.25 (m, 1H), 3.02-2.89 (m, 4H), 2.67 (s, 1H), 2.61 (s, 1H), 2.51 (dd, *J*= 14.3 Hz, *J*= 4.5 Hz, 1H), 2.29 (s, 3H), 2.23 (s, 3H), 1.95 (s, 3H), 1.83 (m, 1H).

^{13}C-NMR (75 MHz, $CDCl_3$): δ 168.2, 152.5, 148.1, 146.2, 144.4, 144.3, 143.3, 139.6, 134.6, 129.7, 129.6, 126.2, 125.6, 123.4, 123.3, 121.6, 118.5, 116.3, 110.7, 110.2, 105.1, 99.4, 98.5, 75.2, 73.3, 61.7, 58.4, 57.9, 56.3, 56.1, 55.1, 54.7, 53.9, 51.9, 45.2, 40.1, 35.6, 33.3, 24.8, 23.3., 14.5, 7.3.

ESI-MS m/z: Calcd.. for $C_{48}H_{49}Cl_3N_4O_{10}S$: 980.3. Found $(M+H)^+$: 981.2

Example 16



31 32

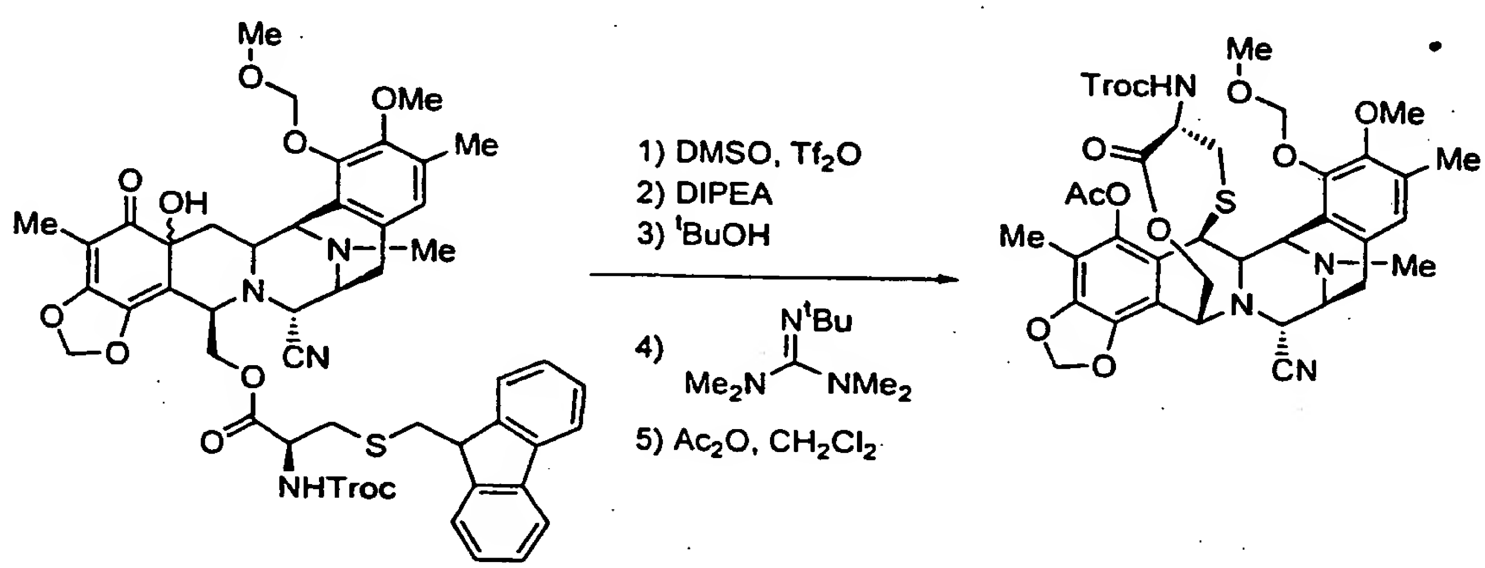
To a solution of **31** (310 mg, 0.32 mmol), in anhydrous CH_2Cl_2 (15 ml) at -10 °C was added a solution of benzeneseleninic anhydride 70 % (165 mg, 0.32 mmol), in anhydrous CH_2Cl_2 (7 ml), *via* cannula, keeping the temperature at -10 °C. The reaction mixture was stirred at -10 °C for 5 min. A saturated solution of sodium bicarbonate (30 ml) was added at this temperature. The aqueous layer was washed with more CH_2Cl_2 (40 ml). The organic layers were dried over sodium sulphate, filtered and concentrated. The crude was purified by flash column chromatography (ethyl acetate/hexane in gradient from 1:5 to 1:1) to obtain 32 (287 mg, 91%, HPLC: 91.3%) as a pale cream yellow solid and as a mixture of two isomers (65:35) which were used in the next step.

^{1}H-NMR (300 MHz, $CDCl_3$): δ (Mixture of isomers) 7.76 (m, 4H), 7.65 (m, 4H), 7.39 (m, 4H), 7.29 (m, 4H), 6.62 (s, 1H), 6.55 (s, 1H), 5.79-5.63 (m, 6H), 5.09 (s, 1H), 5.02 (d, *J*= 6.0 Hz, 1H), 4.99 (d, *J*= 6.0 Hz, 1H), 4.80-4.63 (m, 6H), 4.60 (m, 1H), 4.50 (m, 1H), 4.38 (d, *J*= 12.8 Hz, *J*= 7.5 Hz, 1H), 4.27 (dd, *J*= 12.8 Hz, *J*= 7.5 Hz, 1H), 4.16-3.90 (m, 10H), 3.84 (s, 3H), 3.62 (s, 3H), 3.50 (s, 3H), 3.49 (s, 3H), 3.33-2.83 (m, 14H), 2.45-2.18 (m, 2H), 2.21 (s, 6H), 2.17 (s, 6H), 1.77 (s, 6H), 1.67 (m, 2H).
^{13}C-NMR (75 MHz, $CDCl_3$): δ (Mixture of isomers) 168.6, 168.4, 158.6, 154.8, 152.8, 152.5, 147.3, 147.2, 146.8, 144.1, 144.0, 140.8, 139.7, 137.1, 129.8, 129.3, 128.4, 128.7, 126.5, 125.5, 123.7, 123.6, 123.5,

123.4, 122.2, 121.3, 118.3, 115.8, 115.5, 110.2, 106.9, 103.5, 103.2, 100.1, 99.6, 97.9, 97.7, 93.8, 73.4, 70.9, 69.2, 64.9, 62.5, 59.3, 58.9, 58.4, 56.7, 56.3, 56.2, 55.4, 55.2, 55.1, 54.9, 54.7, 54.3, 54.1, 53.8, 52.8, 45.5, 40.5, 40.0, 39.8, 35.8, 35.5, 33.9, 33.7, 30.1, 28.8, 24.2, 24.1, 21.2, 14.5, 14.4, 12.7, 6.0, 5.7.

ESI-MS m/z: Calcd. for $C_{48}H_{49}Cl_3N_4O_{11}S$: 996.3. Found $(M+H)^+$: 997.2

Example 17



32

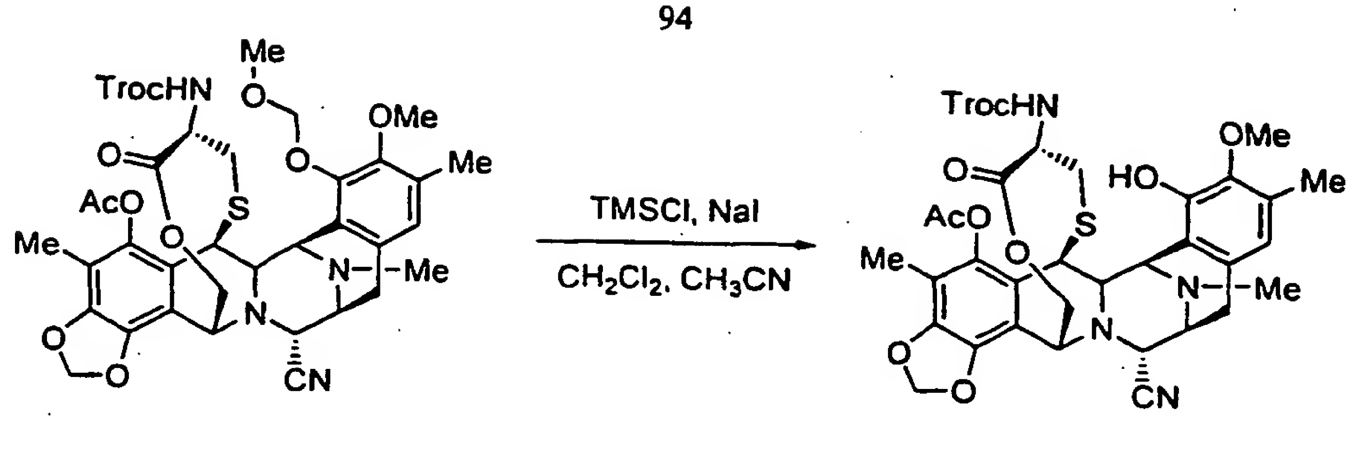
33

The reaction flask was flamed twice, purged vacuum/Argon several times and kept under Argon atmosphere for the reaction. To a solution of DMSO (39.1 ml, 0.55 mmol, 5 equivalents.) in anhydrous CH_2Cl_2 (4.5 ml) was dropwise added triflic anhydride (37.3 ml, 0.22 mmol, 2 equivalents.) at -78 °C . The reaction mixture was stirred at -78 °C for 20 minutes, then a solution of **32** (110 mg, 0.11 mmol, HPLC: 91.3%) in anhydrous CH_2Cl_2 (1 ml, for the main addition and 0.5 ml for wash) at -78 °C was added, *via* cannula. During the addition the temperature was kept at -78 °C in both flasks and the colour changed from yellow to brown. The reaction mixture was stirred at -40 °C for 35 minutes. During this period of time the solution was turned from yellow to dark green. After this time, $^iPr_2NEt$ (153 ml, 0.88 mmol, 8 equivalents.) was dropwise added and the reaction mixture was kept at

0 °C for 45 minutes, the colour of the solution turned to brown during this time. Then t-butanol (41.6 ml, 0.44 mmol, 4 equivalents.) and 2-^{t}Butyl-1,1,3,3-tetramethylguanidine (132.8 ml, 0.77 mmol, 7 equivalents.) were dropwise added and the reaction mixture was stirred at 23 °C for 40 minutes. After this time, acetic anhydride (104.3 ml, 1.10 mmol, 10 equivalents.) was dropwise added and the reaction mixture was kept at 23 °C for 1 hour more. Then the reaction mixture was diluted with CH_2Cl_2 (20ml) and washed with aqueous saturated solution of NH_4Cl (50ml), sodium bicarbonate (50ml), and sodium chloride (50ml). The combined organic layers were dried over sodium sulphate, filtered and concentrated. The residue was purified by flash column chromatography (eluent: ethyl acetate/hexane gradient from 1:3 to 1:2) to afford compound **33** (54 mg, 58%) as a pale yellow solid.

^{1}H-NMR (300 MHz, $CDCl_3$): δ 6.85 (s, 1H), 6.09 (s, 1H), 5.99 (s, 1H), 5.20 (d, *J*= 5.8 Hz, 1H), 5.14 (d, *J*= 5.3 Hz, 1H), 5.03 (m, 1H), 4.82 (d, *J*= 12.2, 1H), 4.63 (d, *J*= 12.0 Hz, 1H), 4.52 (m, 1H), 4.35-4.17 (m, 4H), 3.76 (s, 3H), 3.56 (s, 3H), 3.45 (m, 2H), 2.91 (m, 2H), 2.32 (s, 3H), 2.28 (s, 3H), 2.21 (s, 3H), 2.12 (m, 2H), 2.03 (s, 3H).
^{13}C-NMR (75 MHz, $CDCl_3$): δ 168.5, 167.2, 152.7, 148.1, 147.1, 144.5, 139.6, 139.1, 130.5, 129.0, 123.7, 123.5, 123.3, 118.8, 116.5, 112.1, 100.6, 97.8, 73.3, 60.5, 59.4, 59.2, 58.3, 57.6, 57.4, 56.1, 53.3, 53.1, 40.6, 40.0, 31.0, 22.2, 18.9, 14.4, 8.1.
ESI-MS m/z: Calcd.. for $C_{36}H_{39}Cl_3N_4O_{11}S$: 842.1. Found $(M+H)^+$: 843.1

Example 18



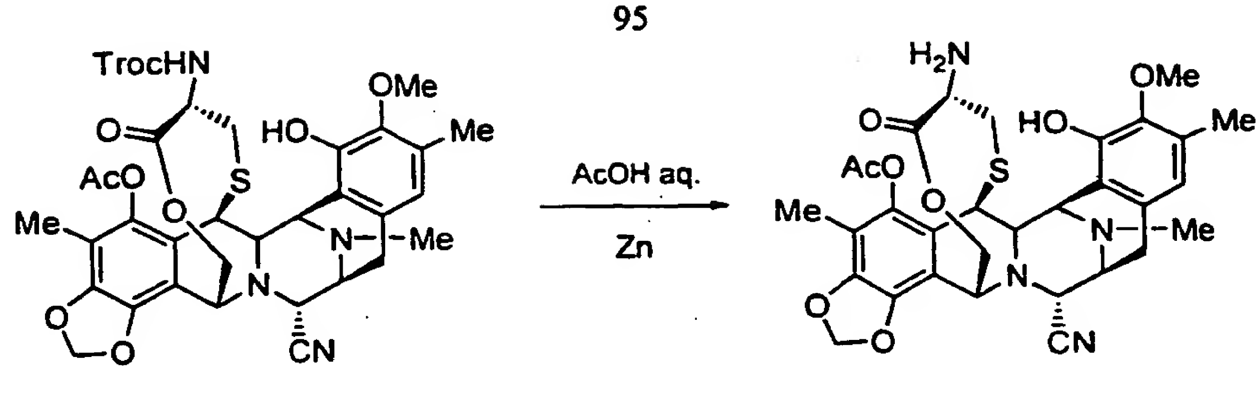
33 34

To a solution of **33** (12 mg, 0.014 mmol)in dry dichloromethane (1.2 ml) and HPLC grade acetonitrile (1.2 ml) was added at 23 °C sodium iodide (21 mg, 0.14 mmol) and freshly distilled (over calcium hydride at atmospheric pressure) trimethylsilyl chloride (15.4 mg, 0.14 mmol). The reaction mixture turned to orange colour. After 15 min the solution was diluted with dichloromethane (10 ml) and was washed with a freshly aqueous saturated solution of $Na_2S_2O_4$ (3 x 10 ml). The organic layer was dried over sodium sulphate, filtered and concentrated. It was obtained compound **34** (13 mg, quantitative) as pale yellow solid which was used without further purification.

^{1}H-NMR (300 MHz, $CDCl_3$): δ 6.85 (s, 1H), 6.09 (s, 1H), 5.99 (s, 1H), 5.27 (d, *J*= 5.8 Hz, 1H), 5.14 (d, *J*= 5.3 Hz, 1H), 5.03 (d, *J*= 11.9 Hz, 1H), 4.82 (d, *J*= 12.2, 1H), 4.63 (d, *J*= 13.0 Hz, 1H), 4.52 (m, 1H), 4.34 (m, 1H), 4.27 (bs, 1H), 4.18 (m, 2H), 3.76 (s, 3H), 3.56 (s, 3H), 3.44 (m, 1H), 3.42 (m, 1H), 2.91 (m, 2H), 2.32 (s, 3H), 2.28 (s, 3H), 2.21 (s, 3H), 2.03 (s, 3H).

ESI-MS m/z: Calcd. for $C_{34}H_{35}N_4O_{10}S$: 798.1. Found $(M+H)^+$: 799.1

Example 19



34 35

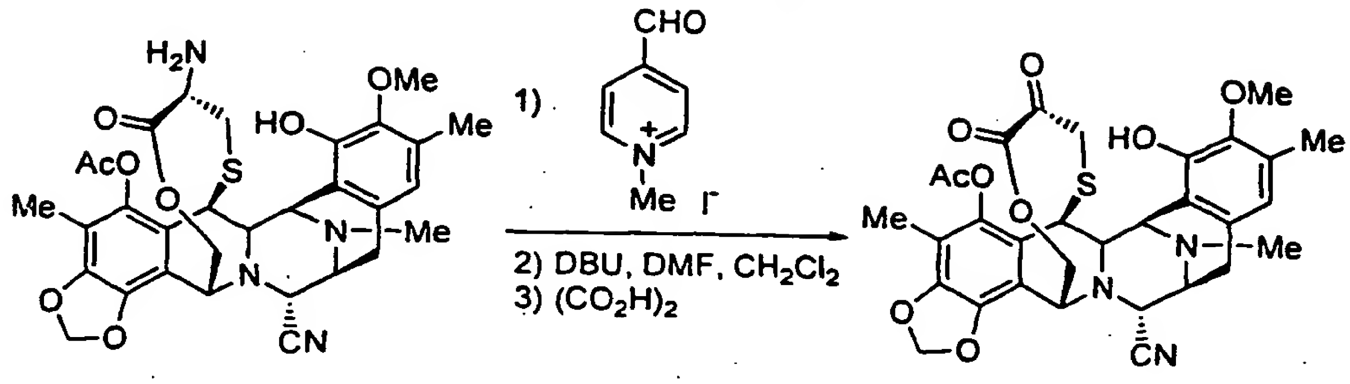
To a solution of **34** (13 mg, 0.016 mmol) in a mixture of acetic acid/H_2O (90:10, 1 ml) was added powder Zinc (5.3 mg, 0.081 mmol) at 23 °C. The reaction mixture was heated at 70 °C for 6 h. After this time, was cooled to 23 °C, diluted with CH_2Cl_2 (20 ml) and washed with aqueous saturated solution of sodium bicarbonate (15 ml) and aqueous solution of Et_3N (15 ml). The organic layer was dried over sodium sulphate, filtered and concentrated. The residue was purified by flash column chromatography with Silica-NH_2 (eluent: ethyl acetate/hexane gradient from 0:100 to 50:50) to afford compound **35** (6.8 mg, 77% for two steps) as a pale yellow solid.

^{1}H-NMR (300 MHz, $CDCl_3$): δ 6.51 (s, 1H), 6.03 (dd, J= 1.3 Hz, J= 26.5 Hz, 2H), 5.75 (bs, 1H), 5.02 (d, J= 11.6 Hz, 1H), 4.52 (m, 1H), 4.25 (m, 2H), 4.18 (d, J= 2.5 Hz, 1H), 4.12 (dd, J= 1.9 Hz, J= 11.5 Hz, 1H), 3.77 (s, 3H), 3.40 (m, 2H), 3.26 (t, J= 6.4 Hz, 1H), 2. 88 (m, 2H), 2.30-2.10 (m, 2H), 2.30 (s, 3H), 2.28 (s, 3H), 2.18 (s, 3H), 2.02 (s, 3H).

^{13}C-NMR (75 MHz, $CDCl_3$): δ 174.1, 168.4, 147.8, 145.4, 142.9, 140.8, 140.1, 131.7, 130.2, 129.1, 128.3, 120.4, 118.3, 117.9, 113.8, 111.7, 101.7, 61.2, 59.8, 59.2, 58.9, 54.4, 53.8, 54.4, 41.3, 41.5, 34.1, 23.6, 20.3, 15.5, 9.4.

ESI-MS m/z: Calcd. for $C_{31}H_{34}N_4O_8S$: 622.7. Found $(M+H)^+$: 623.2.

Example 20



35 36

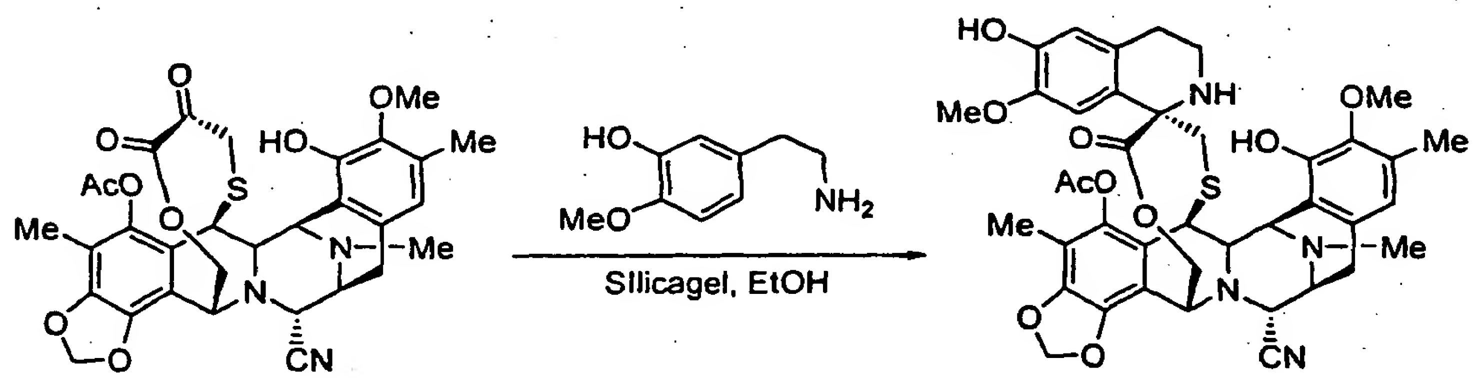
A solution of *N*-methyl pyridine-4-carboxaldehyde iodide (378 mg, 1.5 mmol) in anhydrous DMF (5.8 mL) was treated with anhydrous toluene (2 x 10 mL) to eliminate the amount of water by azeotropic removal of the toluene. A solution of **35** (134 mg, 0.21 mmol), previously treated with anhydrous toluene (2 x 10 mL), in anhydrous CH_2Cl_2 (distilled over CaH_2, 7.2 mL) was added, *via* cannula, at 23 °C to this orange solution. The reaction mixture was stirred at 23 °C for 4 hours. After this time DBU (32.2 □L, 0.21mmol) was dropwise added at 23 °C and it was stirred for 15 minutes at 23 °C. A freshly aqueous saturated solution of oxalic acid (5.8 mL) was added to the reaction mixture and was stirred for 30 minutes at 23 °C. Then the reaction mixture was cooled to 0 °C and $NaHCO_3$ was portionwise added followed by addittion of aqueous saturated solution of $NaHCO_3$. The mixture was extracted with Et_2O. K_2CO_3 was added to the aqueous layer and it was extrated with Et_2O. The combined organic layers were dried over $MgSO_4$ and the solvent was removed under reduced pressure. The crude was purified by flash column chromatography (AcOEt/hexane from 1/3 to 1/1) to afford compound **36** (77 mg, 57%) as pale yellow solid.

^{1}H-NMR (300 MHz, $CDCl_3$): δ 6.48 (s, 1H), 6.11 (d, *J*= 1.3 Hz, 1H), 6.02 (d, *J*= 1.3 Hz, 1H), 5.70 (bs, 1H), 5.09 (d, *J*= 11.3 Hz, 1H), 4.66 (bs, 1H), 4.39 (m, 1H), 4.27 (d, *J*= 5.6 Hz, 1H), 4.21 (d, *J*= 10.5 Hz, 1H), 4.16 (d, *J*= 2.6 Hz, 1H), 3.76 (s, 3H), 3.54 (d, *J*= 5.1 Hz, 1H), 3.42 (d, *J*= 8.5 Hz, 1H), 2.88-2.54 (m, 3H), 2.32 (s, 3H), 2.24 (s, 3H), 2.14 (s, 3H), 2.04 (s, 3H). ^{13}C-NMR (75 MHz, $CDCl_3$): δ 186.7, 168.5, 160.5, 147.1, 146.4,

142.9, 141.6, 140.7, 130.4, 129.8, 121.7 (2C), 120.0, 117.8, 117.1, 113.5, 102.2, 61.7, 61.4, 60.3, 59.8, 58.9, 54.6, 41.6, 36.9, 29.7, 24.1, 20.3, 15.8, 14.1, 9.6.

ESI-MS m/z: Calcd.. for $C_{31}H_{31}N_3O_9S$: 621.7. Found $(M+H)^+$: 622.2

Example 21



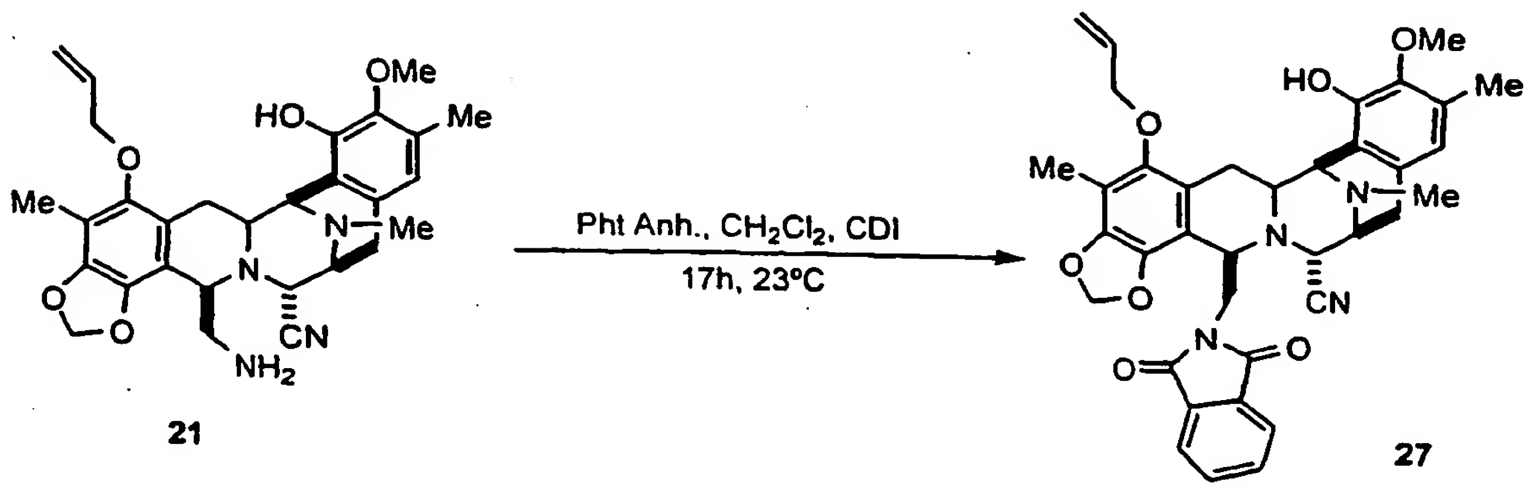
36 **Et-770**

To a solution of **36** (49mg, 0.08 mmol) and 2-[3-hydroxy-4-methoxyphenyl]ethylamine (46.2 mg, 0.27 mmol) in ethanol (2.5 ml) was added silica gel (105 mg) at 23 °C. The reaction mixture was stirred at 23 °C for 14 h. It was diluted with hexane and poured into a column of chromatography (ethyl acetate/hexane from 1/3 to 1/1) to afford **Et-770** (55 mg, 90%) as a pale yellow solid.

^{1}H-NMR (300 MHz, $CDCl_3$): δ 6.60 (s, 1H), 6.47 (s, 1H), 6.45 (s, 1H), 6.05 (s, 1H), 5.98 (s, 1H), 5.02 (d, J=11.4 Hz, 1H), 4.57 (bs, 1H), 4.32 (bs, 1H), 4.28 (d, J= 5.3 Hz, 1H), 4.18 (d, J= 2.5 Hz, 1H), 4.12 (dd, J= 2.1 Hz, J= 11.5 Hz, 1H), 3.78 (s, 3H), 3.62 (s, 3H), 3.50 (d, J= 5.0 Hz, 1H), 3.42 (m, 1H), 3.10 (ddd, J_1= 4.0 Hz, J_2= 10.0 Hz, J_3= 11.0 Hz, 1H), 2.94 (m, 2H), 2.79 (m, 1H), 2.61 (m, 1H), 2.47 (m, 1H), 2.35 (m, 1H), 2.32 (s, 3H), 2.27 (s, 3H), 2.20 (s, 3H), 2.09 (m, 1H), 2.04 (s, 3H).

ESI-MS m/z: Calcd.. for $C_{40}H_{42}N_4O_{10}S$: 770.7. Found $(M+H)^+$: 771.2

Example 22



To a solution of **21** (22 mg, 0.042 mmol) in CH_2Cl_2 (0.8 ml) was added phthalic anhydride (6.44 mg, 0.042 mmol) and the reaction mixture was stirred for 2h at 23 °C. Then, carbonyldiimidazole (1mg, 0.006 mmol) was added and the mixture was stirred at 23 °C for 7h. Then, carbonyldiimidazole (5.86mg, 0.035 ml) was added and the reaction was stirred at 23 °C for an additional 17h. The solution was diluted with CH_2Cl_2 (15 ml) and washed with 0.1 N HCl (15 ml). The organic layer was dried over sodium sulphate, filtered, and the solvent was eliminated under reduced pressure. The residue was purified by flash column chromatography (SiO_2, hexane:ethyl acetate 2:1) to afford **27** (26.4 mg, 96%) as a white solid.

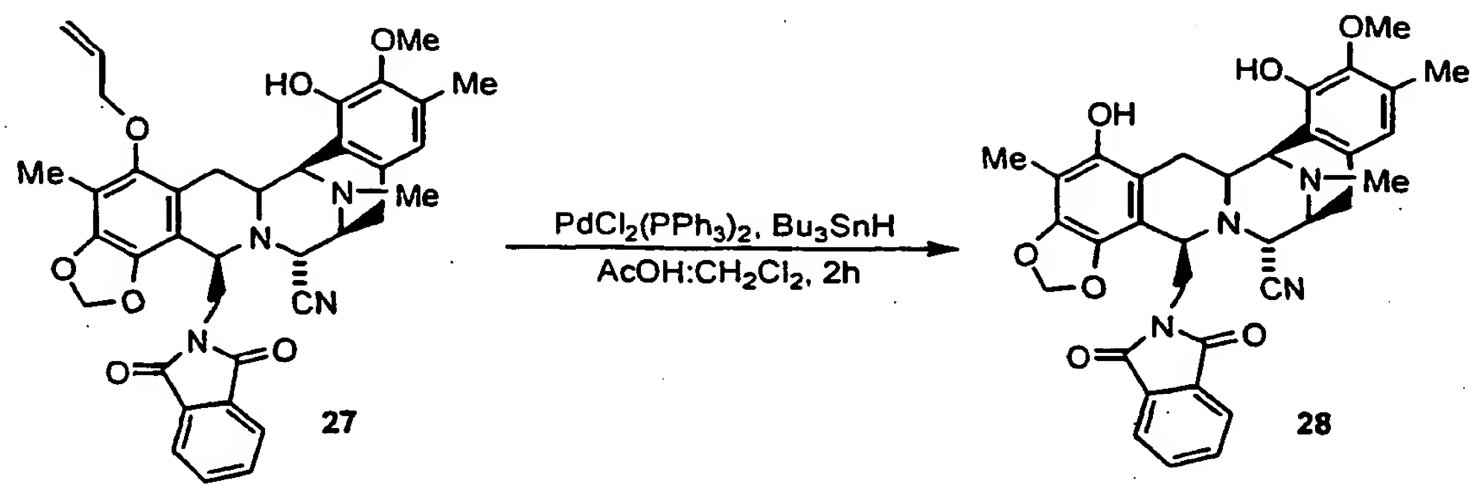
Rf: 0.58 (ethyl acetate).

^1H NMR (300 MHz, $CDCl_3$): δ 7.73–7.64 (m, 4H), 6.40 (s, 1H), 6.12-6.01 (m, 1H), 5.63 (s, 1H), 5.58 (d, J= 1.5 Hz, 1H), 5.37 (dd, J_1= 1.8 Hz, J_2= 17.4 Hz), 5.23 (dd, J_1= 1.8 Hz, J_2= 10.5 Hz, 1H), 5.12 (d, J= 1.5 Hz, 1H), 4.22-4.15 (m, 3H), 4.08 (d, J= 1.8 Hz, 1H), 3.68 (s, 3H), 3.59-3.55 (m 2H), 3.35 (d, J= 8.1 Hz, 1H), 3.27-3.16 (m, 2H), 3.05 (dd, J_1= 8.1 Hz, J_2= 18.3 Hz, 1H), 2.64 (d, J= 18.0Hz, 1H), 2.30 (s, 3H), 2.24 (s, 3H), 2.09 (s, 3H), 1.80 (dd, J_1= 11.4 Hz, J_2= 15 Hz, 1H);

^{13}C NMR (75 MHz, $CDCl_3$): δ 167.7, 148.9, 146.4, 144.2, 142.6, 139.5, 134.0, 133.5, 132.0, 131.0, 128.3, 123.0, 121.3, 120.9, 118.1, 117.5, 116.8, 113.6, 112.4, 100.8, 74.5, 60.6, 60.5, 57.7, 56.6, 55.6, 55.5, 42.3, 41.7, 26.6, 25.5, 15.9, 9.46.

ESI-MS m/z: Calcd. for $C_{37}H_{35}N_4O_7$: 648.79. Found $(M+H)^+$: 649.3.

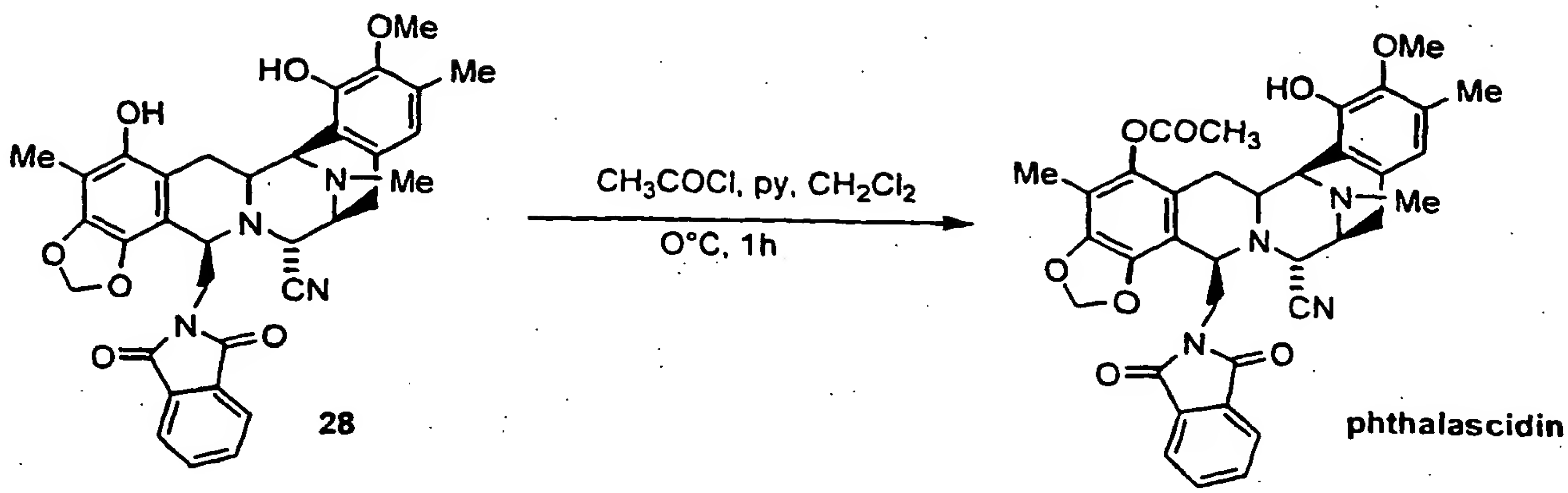
Example 23



To a solution of **27** (26 mg, 0.041 mmol) in CH_2Cl_2 (11 ml), acetic acid (11 ml), $(PPh_3)_2PdCl_2$ (2.36 mg) and Bu_3SnH (28 ml, 0.10 mmol) were added at 23 °C. After stirring at that temperature for 2h the reaction was poured into a pad of flash column (SiO_2, gradient Hex to hexane:ethyl acetate 2:1) to afford **28** (24.7 mg, 99 %) as a white solid.

Rf: 0.33 (hexane:ethyl acetate 2:1).
^1H NMR (300 MHz, $CDCl_3$): δ 7.75-7.70 (m, 2H), 7.69-7.65 (m, 2H), 6.39 (s, 1H), 5.82 (bs, 1H), 5.50 (d, J= 1.5 Hz, 1H), 5.0 (d, J= 1.5 Hz, 1H), 4.45 (bs, 1H), 4.23-4.19 (m, 2H), 4.10-4.09 (m, 1H), 3.73 (s, 3H), 3.60-3.48 (m, 2H), 3.36-3.33 (m, 1H), 3.26-3.20 (m, 1H), 3.14-3.08 (m, 1H), 3.98 (d, J= 14.4 Hz, 1H), 2.61 (d, J= 18.3 Hz, 1H), 2.30 (s, 3H), 2.23 (s, 3H), 2.06 (s, 3H), 1.85 (dd, J_1= 12 Hz, J_2= 15.3 Hz);
^{13}C NMR (75 MHz, $CDCl_3$): δ 167.8, 146.4, 145.1, 143.9, 142.7, 137.1, 133.5, 131.9, 130.8, 128.4, 122.9, 120.8, 118.0, 116.8, 114.0, 113.4, 106.4, 100.4, 60.6, 60.5, 57.8, 56.6, 55.5, 55.2, 42.6, 41.5, 25.6, 25.5, 15.8, 8.9.
ESI-MS m/z: Calcd. for $C_{34}H_{32}N_4O_7$: 608.6. Found $(M+H)^+$: 609.2.

Example 24



To a solution of **28** (357 mg, 0.058 mmol) in CH_2Cl_2 (3 ml), acetyl chloride (41.58 ml, 0.58 mmol) and pyridine (47.3 ml, 0.58 ml) were added at 0 °C. The reaction mixture was stirred for 1h and then, the solution was diluted with CH_2Cl_2 (15 ml) and washed with 0.1 N HCl (15 ml). The organic layer was dried over sodium sulphate, filtered, and the solvent was eliminated under reduced pressure. The residue was purified by flash column chromatography (RP-18, $CH_3CN:H_2O$ 60:40) to afford phthalascidin (354 mg, 94%) as a white solid.

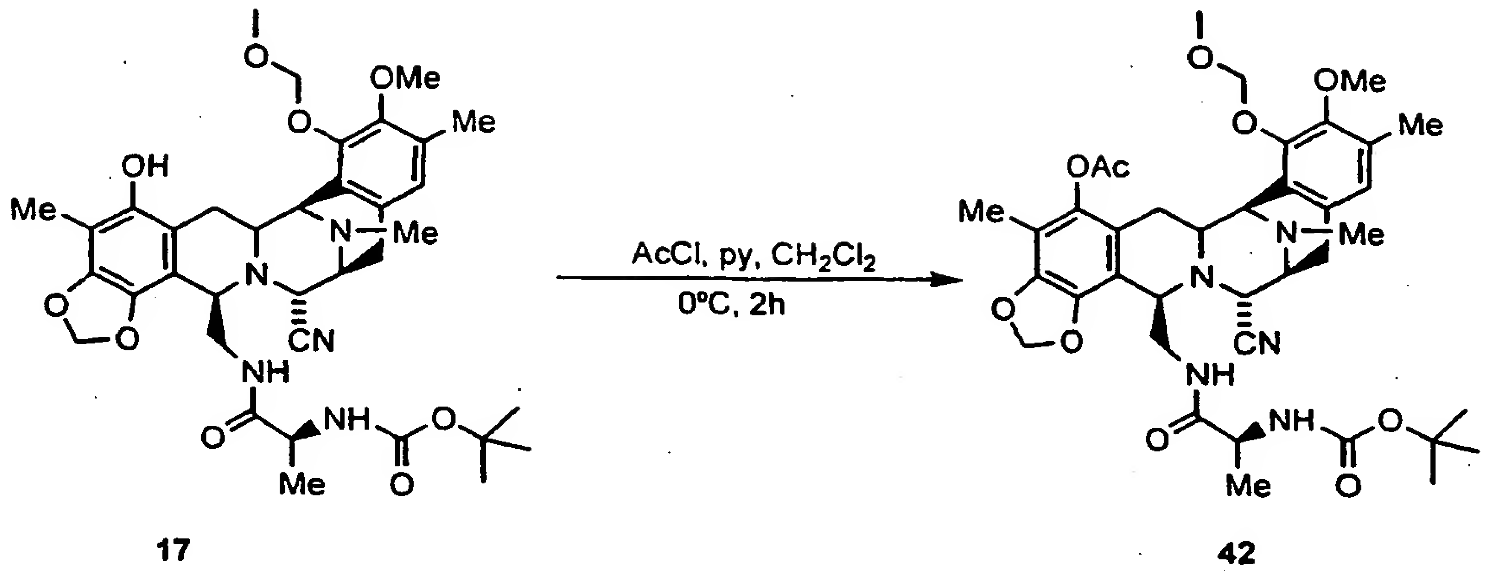
Rf: 0.37 ($CH_3CN:H_2O$ 7:3, RP-18).

^{1}H NMR (300 MHz, $CDCl_3$): δ 7.72–7.68 (m, 2H), 7.67-7.63 (m, 2H), 6.38 (s, 1H), 5.69 (d, *J*= 1.2 Hz, 1H), 5.64 (d, *J*= 1.2Hz, 1H), 5.30 (bs, 1H), 4.25-4.21 (m, 2H), 4.02 (d, *J*= 2.1 Hz, 1H), 3.64-3.62 (m, 5H), 3.33 (d, *J*= 8.4 Hz, 1H), 3.21-3.16 (m, 1H), 3.02 (dd, J_1= 8.1 Hz, J_2= 18 Hz, 1H), 2.76 (dd, J_1= 1.8 Hz, J_2= 15.6 Hz, 1H), 2.63 (d, *J*= 17.7 Hz, 1H), 2.29 (s, 3H), 2.28 (s,3H), 2.21 (s, 3H), 2.0 (s, 3H), 1.73 (dd, J_1= 12.0 Hz, J_2= 15.3 Hz, 1H))

^{13}C NMR (75 MHz, $CDCl_3$)): δ 168.5, 167.6, 146.2, 144.2, 142.5, 141.0, 140.5, 133.4, 131.8, 130.7, 128.2, 120.9, 120.8, 117.9, 116.4, 113.6, 101.1, 60.4, 60.0, 57.0, 56.3, 55.6, 55.4, 41.6, 41.5, 26.5, 25.2, 20.2, 15.7, 9.4.

ESI-MS m/z: Calcd. for $C_{36}H_{34}N_4O_8$: 650. Found $(M+H)^+$: 651.2.

Example 25



To a solution of **17** (300 mg, 0.432 mmol) in CH_2Cl_2 (2 ml), acetyl chloride (30.7 ml, 0.432 mmol) and pyridine (34.9 ml, 0.432 mmol) were added at 0 °C. The reaction mixture was stirred for 2h at that temperature and then, the solution was diluted with CH_2Cl_2 (15 ml) and washed with 0.1 N HCl (15 ml). The organic layer was dried over sodium sulphate, filtered, and the solvent was eliminated under reduced pressure to afford **42** (318 mg, 100%) as a white solid that was used in subsequent reactions with no further purification.

Rf: 0.5 (ethyl acetate:methanol 5:1).

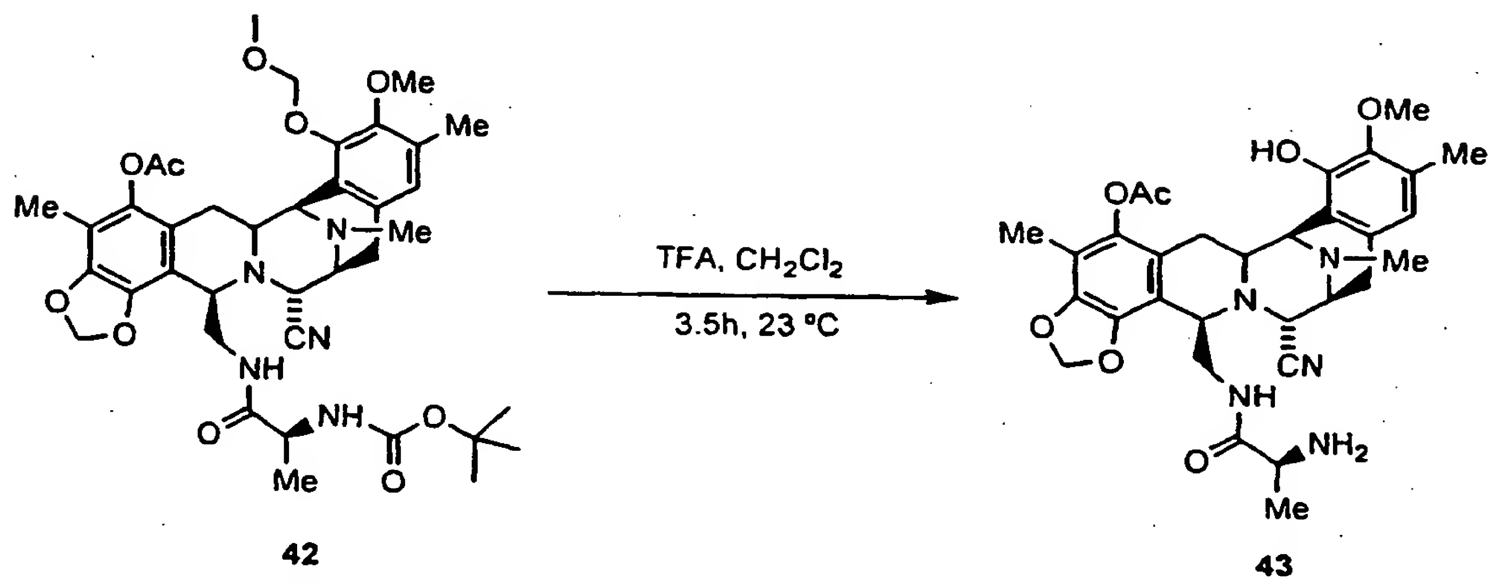
^{1}H NMR (300 MHz, $CDCl_3$). δ 6.66 (s, 1H), 5.93 (d, J= 1.2 Hz, 1H), 5.83 (d, J= 1.2 Hz, 1H), 5.42 (t, J= 6.6 Hz, 1H), 5.07 (d, J= 5.7 Hz, 1H), 4.98 (d, J= 5.7 Hz, 1H), 4.16 (d, J= 1.8 Hz, 1H), 4.11 (d, J= 2.7 Hz, 1H), 3.98 (bs, 1H), 3.73-3.61 (m, 2H), 3.64 (s, 3H), 3.52-3.48 (m, 1H), 3.50 (s, 3H), 3.33 (d, J= 9.6 Hz, 1H), 3.17-3.14 (m, 1H), 2.97-2.87 (m, 1H), 2.75-2.70 (d, J= 16.8 Hz, 1H), 2.26 (s, 6H), 2.16 (s, 3H), 1.96 (s, 3H), 1.70 (dd, J_1= 11.7 Hz, J_2= 15.6 Hz, 1H), 1.33 (s, 9H), 0.59 (d, J= 6.0 Hz, 3H).

^{13}C NMR (75 MHz, $CDCl_3$)): δ 172.0, 168.3, 162.3, 148.2, 144.4, 140.4, 140.2, 130.9, 130.5, 125.3, 123.4, 120.8, 117.6, 112.7, 111.7, 101.4,

99.1, 79.2, 59.5, 58.8, 57.5, 57.4, 56.4, 55.5, 55.0, 41.3, 39.0, 28.2, 26.4, 24.6, 19.9, 18.4, 15.4, 9.1.

ESI-MS m/z: Calcd. for $C_{38}H_{49}N_5O_{10}$: 735.82. Found $(M+H)^+$: 736.3.

Example 26



To a solution of **42** (318 mg, 0.432 mmol) in CH_2Cl_2 (2.16 mmol), trifluoroacetic acid (1.33 ml, 17.30 mmol) was added and the reaction mixture was stirred for 3.5h at 23 °C. The reaction was quenched at 0 °C with saturated aqueous sodium bicarbonate (60 ml) and extracted with CH_2Cl_2 (2 x 70 ml). The combined organic layers were dried (sodium sulphate) and concentrated *in vacuo*. The residue was purified by flash column chromatography (SiO_2, ethyl acetate:methanol 20:1) to afford **43** (154 mg, 60%) as a white solid.

Rf: 0.22 (ethyl acetate:methanol 5:1).

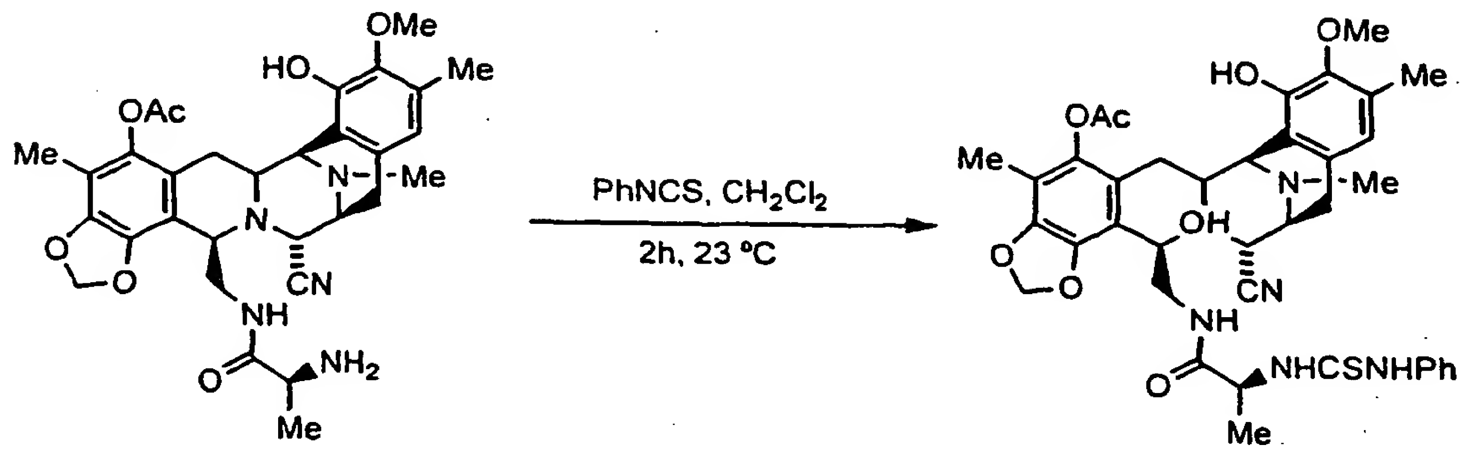
^1H NMR (300 MHz, $CDCl_3$). δ 6.47 (s, 1H), 6.22 (bs, 1H), 5.95 (d, J= 1.2 Hz, 1H), 5.88 (d, J= 1.2 Hz, 1H), 4.08-4.06 (m, 2H), 4.01 (bs, 1H), 3.69 (s, 3H), 3.49 (d, J= 3.6 Hz, 1H), 3.33 (d, J= 8.1 Hz, 1H), 3.26-3.22 (m, 1H), 2.95 (dd, J_1= 8.1 Hz, J_2= 18 Hz, 1H), 2.80-2.76 (m, 2H), 2.58 (d, J=18Hz, 1H), 2.29 (s, 3H), 2.27 (s, 3H), 2.21 (s, 3H), 1.96 (s, 3H), 1.77 (dd, J_1= 12.3 Hz, J_2= 15.6 Hz, 1H), 0.90 (d, J=6.9 Hz, 3H).

^{13}C NMR (75 MHz, $CDCl_3$)): δ 174.8, 169.0, 146.8, 144.4, 142.8, 140.5, 140.2, 131.1, 128.8, 120.8, 120.5, 117.1, 112.9, 111.6, 101.5, 60.3,

59.0, 56.5, 56.3, 55.6, 55.1, 50.2, 41.6, 39.5, 26.8, 26.3, 24.9, 20.2, 15.4, 9.2.

ESI-MS m/z: Calcd. for $C_{31}H_{37}N_5O_7$: 591.65. Found $(M+H)^+$: 592.3.

Example 27



43 **44**

To a solution of **43** (154 mg, 0.26 mmol) in CH_2Cl_2 (1.3 ml), phenyl isothiocyanate (186 ml, 1.56 mmol) was added and the mixture was stirred at 23° C for 2h. The reaction was concentrated *in vacuo* and the residue was purified by flash column chromatography (SiO_2, gradient Hexane to hexane:ethyl acetate 1:1) to afford **44** (120 mg, 63 %) as a white solid.

Rf: 0.41 (ethyl acetate:methanol 5:1).

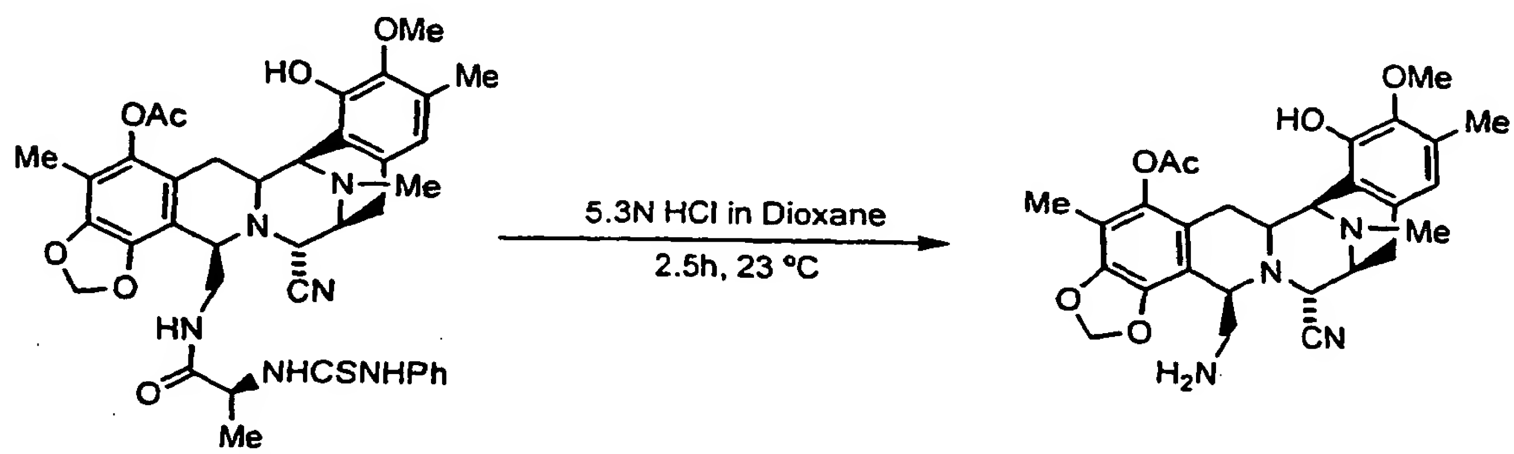
^1H NMR (300 MHz, $CDCl_3$). δ 8.17 (s, 1H), 7.49-7.44 (m, 3H), 7.31-7.24 (m, 3H), 7.05 (d, J= 6.9 Hz, 1H), 5.98 (d, J= 1.2 Hz, 1H), 5.87 (d, J= 1.2 Hz, 1H), 5.52 (bs, 1H), 4.54 (t, J= 6.6 Hz, 1H), 4.15 (d, J= 2.1 Hz, 1H), 4.03 (d, J= 2.7 Hz, 2H), 3.80 (bs, 1H), 3.66 (s, 3H), 3.40 (bs, 1H), 3.32 (d, J= 7.8 Hz, 1H), 3.16 (d, J= 11.7 Hz, 1H), 2.82-2.61 (m, 3H), 2.29 (s, 3H), 2.20 (s, 3H), 2.01 (s, 3H), 1.99 (s, 3H), 1.80 (dd, J_1= 12.0 Hz, J_2= 15.9 Hz, 1H), 0.62 (d, J= 6.0 Hz, 3H).

^{13}C NMR (75 MHz, $CDCl_3$) δ 178.5, 171.9, 168.7, 146.7, 144.5, 142.6, 140.6, 140.3, 136.3, 131.0, 129.9, 128.9, 126.7, 124.4, 120.9, 120.6,

117.7, 116.6, 112.7, 111.9, 101.4, 60.4, 58.7, 57.5, 56.1, 55.7, 55.1, 53.3, 41.4, 38.8, 26.3, 24.4, 20.2, 18.1, 15.3, 9.2.

ESI-MS m/z: Calcd. for $C_{38}H_{42}N_6O_7S$: 726.3. Found $(M+H)^+$: 727.3.

Example 28



44 45

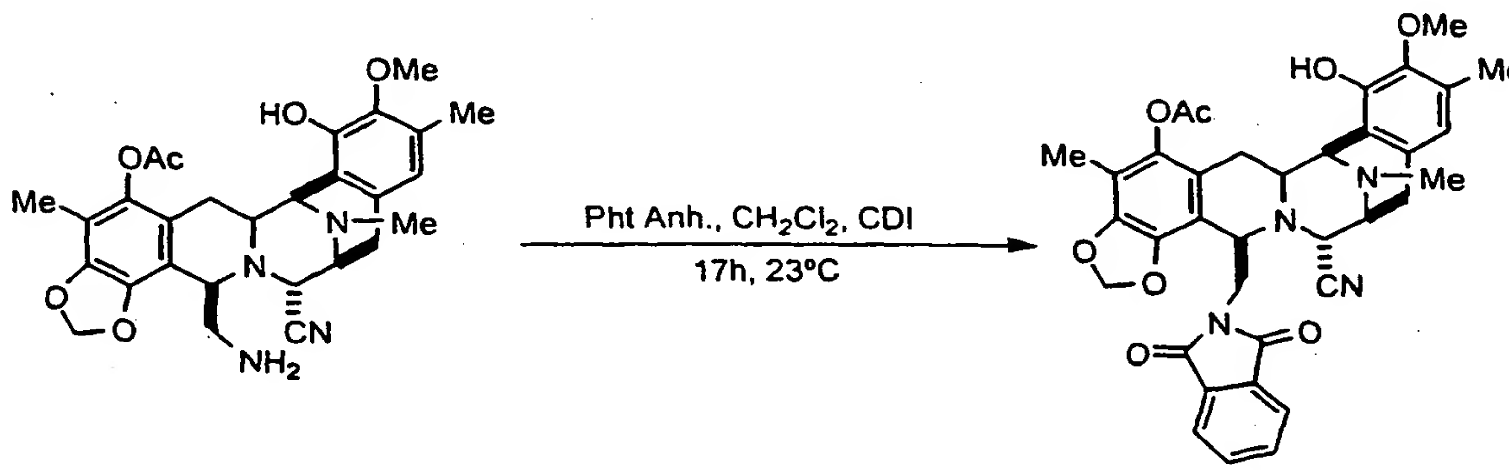
To a solution of **44** (120 mg, 0.165 mmol) in dioxane (0.9 ml), 5.3N HCl/dioxane (1.8 ml) was added and the reaction was stirred at 23 °C for 2.5h. Then, CH_2Cl_2 (10 ml) and H_2O (5 ml) were added to this reaction and the organic layer was decanted. The aqueous phase was basified with saturated aq sodium bicarbonate (20 ml) (pH = 8) at 0 °C and then, extracted with CH_2Cl_2 (2x15 ml). The combined organic extracts were dried (sodium sulphate), and concentrated *in vacuo* to afford **45** (75 mg, 87%) as a white solid that was used in subsequent reactions with no further purification.

Rf: 0.23 (ethyl acetate:methanol 5:1).

^1H NMR (300 MHz, $CDCl_3$): δ 6.43 (s, 1H), 5.94 (d, J= 1.2 Hz, 1H), 5.87 (d, J= 1.2Hz, 1H), 4.10 (d, J= 2.1 Hz, 1H), 3.98 (d, J= 2.4 Hz, 1H), 3.91 (bs, 1H), 3.69 (s, 3H), 3.34-3.25 (m, 2H), 3.05 (dd, J_1= 1.8 Hz, J_2= 8.1 Hz, 1H), 2.80-2.73 (m, 3H), 2.46 (d, J= 18 Hz, 1H), 2.30 (s, 3H), 2.28 (s,3H), 2.20 (s, 3H), 1.98 (s, 3H), 1.79 (dd, J_1= 12.6 Hz, J_2= 16.2 Hz, 1H);

^{13}C NMR (75 MHz, $CDCl_3$)): δ 168.7, 146.7, 144.4, 142.9, 140.4, 130.4, 128.9, 121.1, 120.8, 117.8, 116.8, 113.6, 111.5, 101.4, 67.6, 60.5, 59.8, 58.4, 56.6, 55.8, 55.3, 43.6, 41.8, 31.3, 25.6, 20.2, 15.6, 9.2.
ESI-MS m/z: Calcd. for $C_{28}H_{32}N_4O_6$: 520.58. Found $(M+H)^+$: 521.3.

Example 29



45

Phtalascidin

To a solution of **45** (10 mg, 0.02 mmol) in CH_2Cl_2 (0.4 ml) was added phthalic anhydride (2.84 mg, 0.02 mmol) and the reaction mixture was stirred for 2 h at 23 °C. Then, carbonyldiimidazole (0.5 mg, 0.003 mmol) was added and the mixture was stirred at 23 °C for 7h. Then, carbonyldiimidazole (2.61 mg, 0.016 mmol) was added and the reaction was stirred at 23 °C for an additional 17h. The solution was diluted with CH_2Cl_2 (10 ml) and washed with 0.1 N HCl (5 ml). The organic layer was dried over sodium sulphate, filtered, and the solvent was eliminated under reduced pressure. The residue was purified by flash column chromatography (RP-18, $CH_3CN:H_2O$ 60:40) to afford phthalascidin (11.7 mg, 93%) as a white solid.

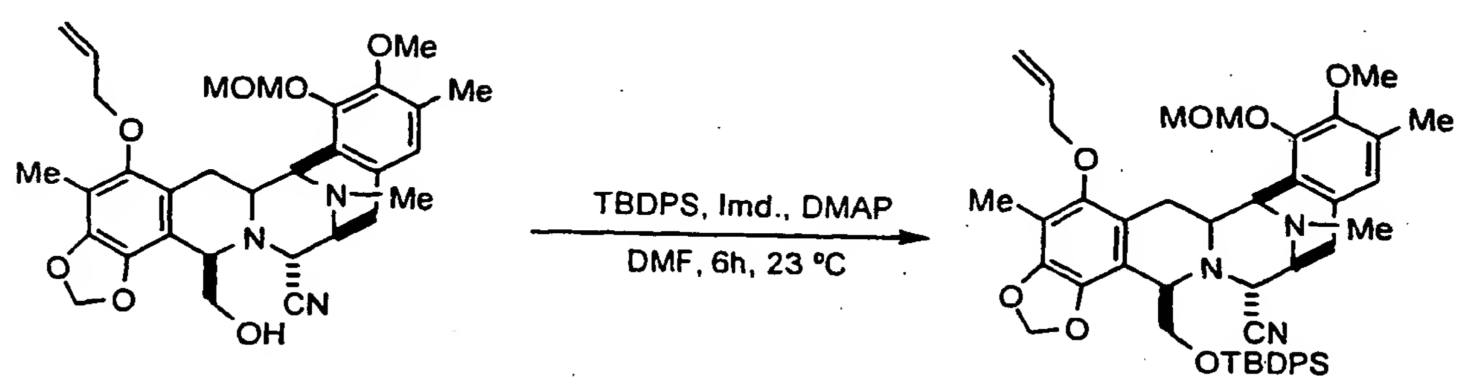
Rf: 0.37 ($CH_3CN:H_2O$ 7:3, RP-18).
^1H NMR (300 MHz, $CDCl_3$): δ 7.72–7.68 (m, 2 H), 7.67-7.63 (m, 2 H), 6.38 (s, 1H), 5.69 (d, J= 1.2 Hz, 1H), 5.64 (d, J= 1.2 Hz, 1H), 5.30 (bs, 1H), 4.25-4.21 (m, 2 h), 4.02 (d, J= 2.1 Hz, 1H), 3.64-3.62 (m, 5H), 3.33 (d, J= 8.4 Hz, 1H), 3.21-3.16 (m, 1H), 3.02 (dd, J_1= 8.1 Hz, J_2= 18 Hz, 1H),

2.76 (dd, J_1= 1.8 Hz, J_2= 15.6 Hz, 1H), 2.63 (d, J= 17.7 Hz, 1H), 2.29 (s, 3H), 2.28 (s,3H), 2.21 (s, 3H), 2.0 (s, 3H), 1.73 (dd, J_1= 12.0 Hz, J_2= 15.3 Hz, 1H)).

^{13}C NMR (75 MHz, $CDCl_3$)): δ 168.5, 167.6, 146.2, 144.2, 142.5, 141.0, 140.5, 133.4, 131.8, 130.7, 128.2, 120.9, 120.8, 117.9, 116.4, 113.6, 101.1, 60.4, 60.0, 57.0, 56.3, 55.6, 55.4, 41.6, 41.5, 26.5, 25.2, 20.2, 15.7, 9.4.

ESI-MS m/z: Calcd. for $C_{36}H_{34}N_4O_8$: 650. Found $(M+H)^+$: 651.2.

Example 30



25

26

To a solution of **25** (18 mg, 0.032 mmol) in DMF (0.05 ml), cat. DMAP (0.5 mg, 0.004 mmol), imidazole (5 mg, 0.08 mmol) and *tert*-Butyldiphenylsilyl chloride (12.5 ml, 0.048 mmol) were added at 0 °C and the reaction mixture was stirred for 6h at 23 °C. Water (10 ml) was added at 0 °C and the aqueous phase was extracted with hexane:ethyl acetate 1:10 (2 x 10 mmol). The organic layer was dried (sodium sulphate), filtered, and the solvent was removed under reduced pressure. The crude was purified by flash column chromatography (SiO_2, hexane:ethyl acetate 3:1) to afford **26** (27 mg, 88 %) as a white solid.

Rf: 0.29 (hexane:ethyl acetate 3:1).

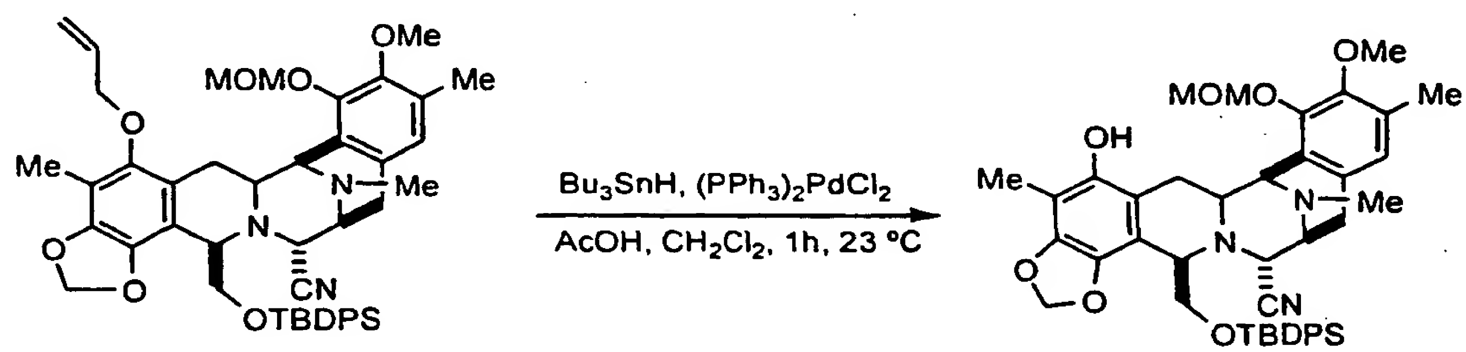
^1H NMR (300 MHz, $CDCl_3$) δ 7.61-7.58 (m, 2 h), 7.42-7.28 (m, 8H), 6.71 (s, 1H), 6.19-6.02 (m, 1H), 5.78 (d, J= 1.2 Hz, 1H), 5.64 (d, J= 1.2 Hz,

1H), 5.40 (dd, J_1= 1.2 Hz, J_2= 17.1 Hz, 1H), 5.27 (dd, J_1= 1.2 Hz, J_2= 10.2 Hz, 1H), 5.13 (s, 2 h), 4.45 (d, J= 2.4 Hz, 1H), 4.24 (d, J= 2.1 Hz, 1H), 4.17-4.06 (m, 3H), 3.75 (s, 3H), 3.64 (dd, J_1= 2.4 Hz, J_2= 9.9 Hz, 1H), 3.59 (s, 3H), 3.42-3.21 (m, 4H), 3.10 (dd, J_1= 8.1 Hz, J_2= 17.7 Hz, 1H), 2.70 (d, J= 17.7 Hz, 1H), 2.33 (s, 3H), 2,26 (s, 3H), 2.11 (s, 3H), 2.08-1.89 (m, 1H), 0.87 (s, 9H);

^{13}C NMR (75 MHz, $CDCl_3$): δ 148.5, 148.3, 148.1, 144.0, 139.0, 135.6, 135.4, 133.8, 133.1, 132.6, 130.5, 130.3, 129.6, 129.4, 127.5, 127.4, 125.1, 124.3, 121.6, 118.5, 117.5, 112.9, 111.7, 100.8, 99.2, 74.0, 67.7, 61.5, 59.6, 59.0, 57.7, 57.1, 55.4, 41.6, 29.6, 26.6, 25.5, 18.8, 15.8, 9.2.

ESI-MS m/z: Calcd. for $C_{47}H_{55}N_3O_7Si$: 801.3. Found $(M+H)^+$: 802.3.

Example 31



26 ET-11

To a solution of **26** (7 mg, 0.0087 mmol) in CH_2Cl_2 (0.15 ml), acetic acid (2.5 ml, 0.044 mmol), $(PPh_3)_2PdCl_2$ (0.5 mg, 6.96 x 10^{-4} mmol) and Bu_3SnH (3.5 ml, 0.013 mmol) were added at 23 °C. The reaction mixture was stirred at that temperature for 1h. The solution was diluted with a mixture of hexane:ethyl acetate 5:1 (0.5 ml) and poured into a pad of flash column (SiO_2, gradient 5:1 to 1:1 hexane:ethyl acetate) affording **ET-11** (5 mg, 75 %) as a white solid.

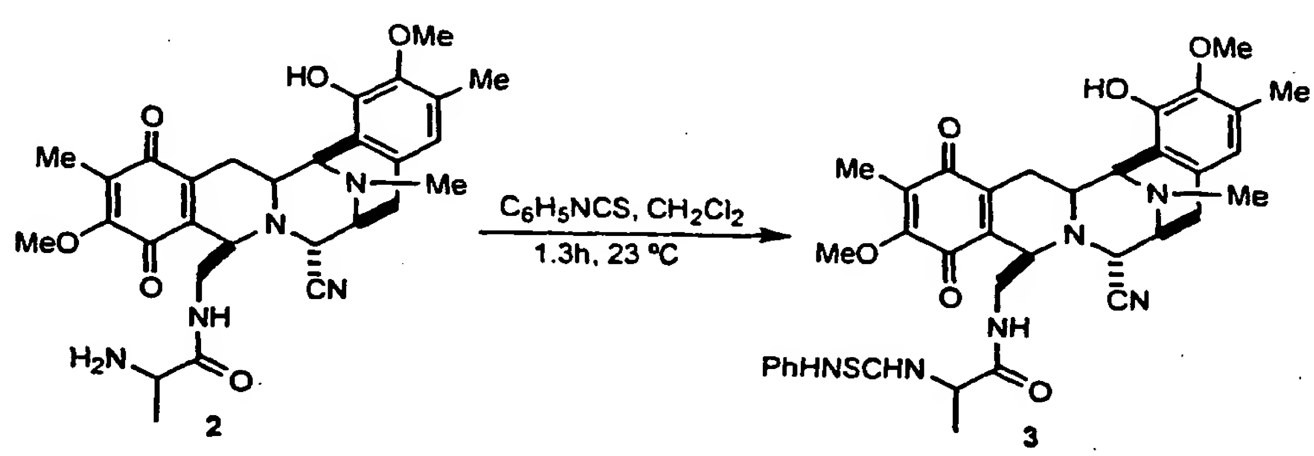
Rf: 0.36 (hexane:ethyl acetate 1:5, silica).

^{1}H NMR (300 MHz, $CDCl_3$): δ 7.56 (m, 2 h), 7.41-7.25 (m, 8H), 6.67 (s, 1H), 5.72 (d, J= 1.0 Hz, 1H), 5.58 (d, J= 1.0 Hz, 1H), 5.51 (s, 1H), 5.38 (d, J= 5.75 Hz, 1H), 5.16 (d, J= 5.7 Hz, 1H), 4.57 (d, J= 2.9 Hz, 1H), 4.21 (m, 1H), 4.09 (m, 1H), 3.72 (s, 3H), 3.71 (s, 3H), 3.68 (dd, J_1= 2.1 Hz, J_2= 10.4 Hz, 1H), 3.38-3.26 (m, 3H), 3.11 (dd, J_1= 2.5 Hz, J_2= 15.7 Hz, 1H), 3.01 (dd, J_1= 8.9 Hz, J_2= 17.9 Hz, 1H), 2.70 (d, J= 17.9 Hz, 1H), 2.31 (s, 3H), 2.25 (s, 3H), 2.06 (s, 3H), 1.89 (dd, J_1= 12.1 Hz, J_2= 15.7 Hz, 1H), 0.9 (s, 9H).);

^{13}C NMR (75 MHz, $CDCl_3$): δ 149.0, 147.4, 145.3, 144.3, 136.3, 135.7, 135.4, 133.2, 130.9, 130.5, 129.6, 129.5, 127.5, 125.0, 118.6, 112.5, 112.1, 105.7, 100.5, 99.8, 68.5, 61.5, 59.7, 58.8, 57.7, 56.9, 56.5, 55.4, 41.7, 26.6, 26.2, 25.5, 18.9, 15.8, 14.2, 8.7.

ESI-MS m/z: Calcd. for $C_{44}H_{51}N_3O_7Si$: 761. Found $(M+H)^+$: 762.

Example 32



A solution of **2** (3.0 g, 5.46 mmol) and phenyl isothiocyanate (3.92mL, 32.76 mmol) in CH_2Cl_2 (27 ml) was stirred at 23° C for 1.5h. The reaction mixture was partitioned between CH_2Cl_2 (10 ml) and H_2O (5 ml). The organic layer was dried over sodium sulphate, filtered and concentrated. The residue was purified by flash column chromatography (SiO_2, gradient Hex to 2:3 hexane:ethyl acetate) to give **3** (3.29 g, 88%) as a yellow solid.

Rf: 0.27 (ACN:H_2O 3:2, RP-C18);

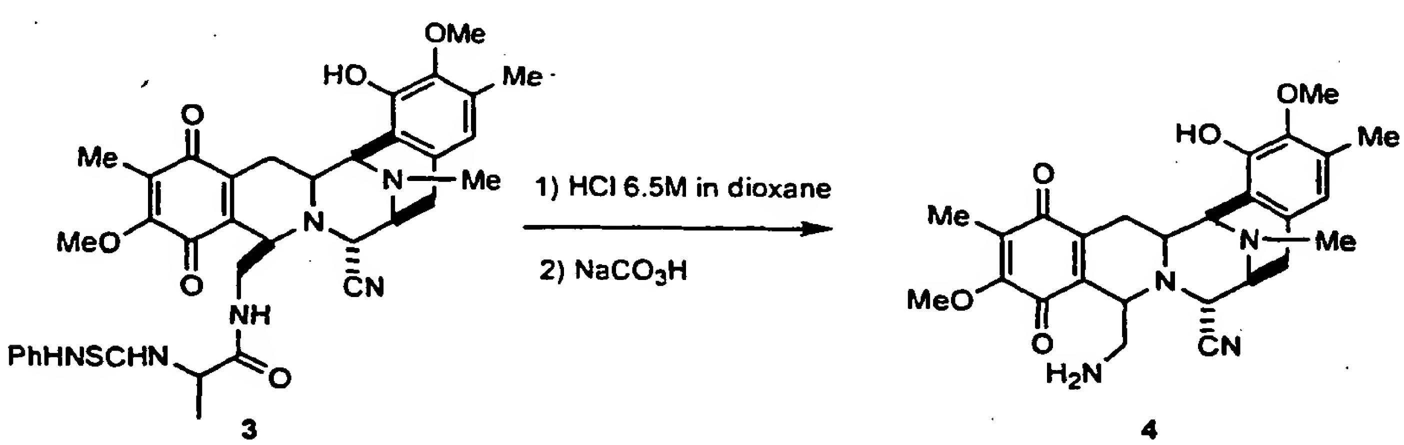
^{1}H NMR (300 MHz, $CDCl_3$): δ 7.77 (bs, 1H), 7.42-7.11 (m, 5H), 6.65 (d, 1H), 6.29 (s, 1H), 5.6-5.5 (m, 1H), 4.19-4.14 (m, 2 h), 4.08 (d, 1H), 3.92 (s, 3H), 3.87-3.65 (m, 6H), 3.77 (s, 3H), 3.37-2.98 (m, 8H), 2.50 (d, 1H), 2.31 (s, 3H), 2.20 (s, 3H), 1.96 (d, 1H), 1.87 (s, 3H), 1.81-1.75 (m, 1H), 0.96 (d, 3H);

^{13}C NMR (75 MHz,

$CDCl_3$): δ 185.7, 180.9, 178.9, 172.0, 155.7, 147.1, 143.2, 142.4, 136.0, 135.1, 130.5, 1 29.9, 129.3, 128.5, 126.9, 124.4, 120.2, 117.4, 116.3, 77.1, 60.9, 58.6, 56.2, 55.8, 55.0, 54.6, 53.5, 41.7, 40.3, 25.1, 24.5, 18.4, 15.8, 8.7

ESI-MS m/z: Calcd. for $C_{36}H_{40}N_6O_6S$: 684.8. Found $(M+H)^+$: 685.2.

Example 33



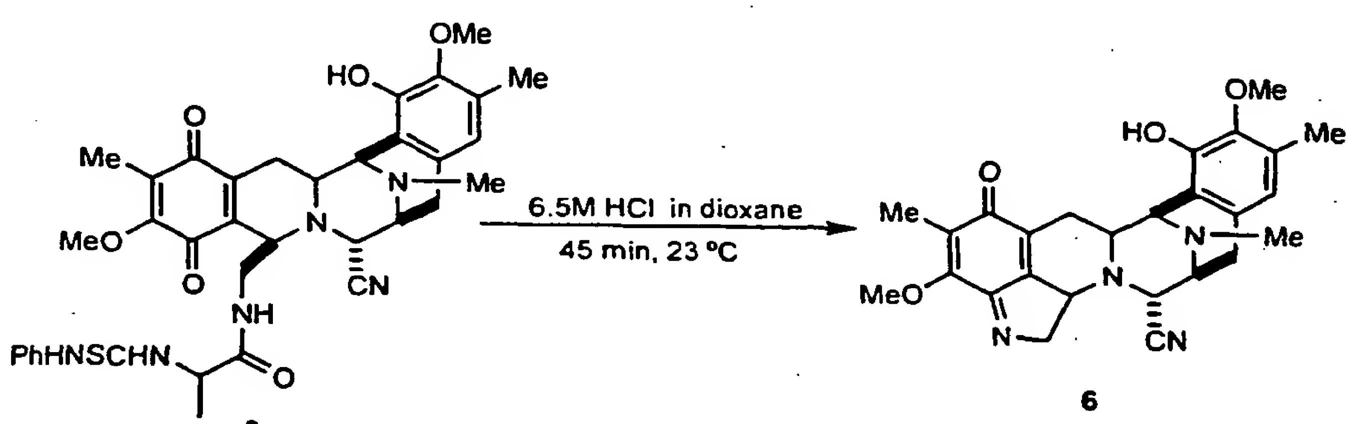
A solution of **3** (0.143 g, 0.208 mmol) in 6.5 M HCl/dioxane (150 ml) was stirred at 23 °C for 6h. Then, toluene (3 ml) was added to this reaction and the organic layer was decanted. The residue was partitioned between saturated aqueous sodium bicarbonate (3 ml) and $CHCl_3$ (3x3 ml) The organic layers were dried and concentrated to afford title compound as a mixture of **4** and **6** (**4**:**6** 90:10) which slowly cyclizes to **6** on standing.

Rf: 0.4 (ethyl acetate:methanol5:1, silica);

^{1}H NMR (300 MHz, $CDCl_3$): δ 6.45 (s, 1H), 4.16 (m, 1H), 4.02 (d, 1H), 3.96 (s, 3H), 3.79 (m, 2 h), 3.75 (s, 3H), 3.35 (m, 1H), 3.20-3.00 (m, 3H), 2.87 (d, 1H), 2.75 (d, 1H), 2.43 (d, 1H), 2.34 (s, 3H), 2.30 (s, 3H), 1.93 (s, 3H), 1.72-1.5 (m, 3H).

ESI-MS m/z: Calcd. for $C_{26}H_{30}N_4O_5$: 478.5. Found $(M+H)^+$: 479.2

Example 34



3

6

A solution of **3** (0.143 g, 0.208 mmol) in 6.5M HCl/dioxane (150 ml) was stirred at 23 °C for 1h. Evaporation of the solvent gave a residue which was purified by flash column chromatography (ethyl acetate/methanol/triethylamine 100:25:0.1) to give **6** (80 mg, 83%) as a yellow solid.

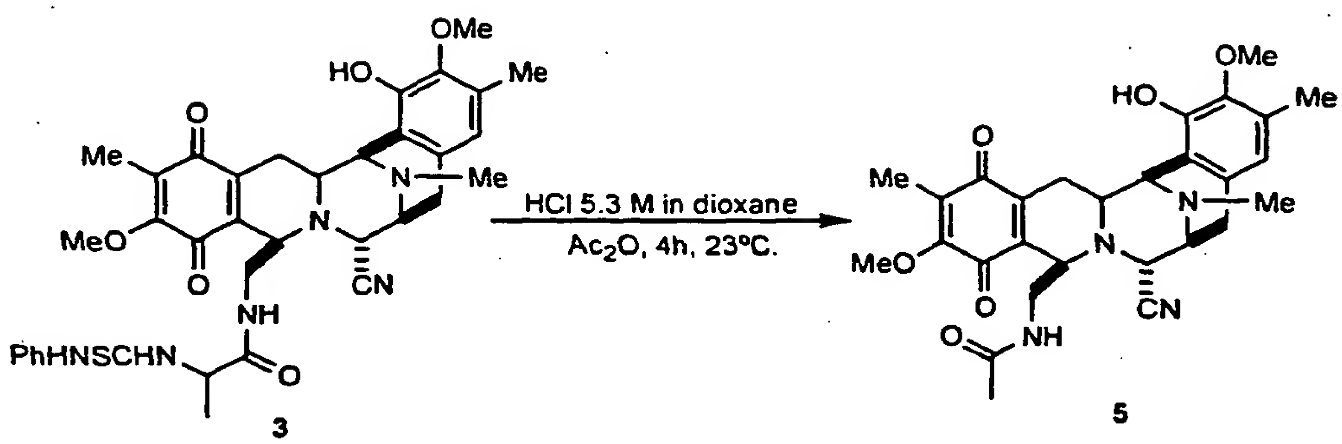
Rf: 0.26 (ACN:H_2O 3:2, RP-C18);

^{1}H NMR (500 MHz, $CDCl_3$): δ 6.46 (s, 1H), 5.9 (bs, 1H) 4.67 (dd, J=18.3 Hz, J= 7.8 Hz, 1H), 4.24 (d, 1H), 4.16 (s, 3H), 3.93 (d, J=2.7 Hz, 1H), 3.8 (m, 2 h), 3.77 (s, 3H), 3.45 (m, 2 h), 3.08 (dd, J=17.9 Hz, J=3.6 Hz, 1H), 2.78 (m, 1H), 2.55 (d, 1H), 2.3 (m, 1H), 2.3 (s, 3H), 2. 28 (s, 3H), 1.90 (s, 3H);

^{13}C NMR (75 MHz,$CDCl_3$): δ 186.2, 162.1, 154.9, 146.9, 145.3, 143.0, 130.1, 129.4, 128,1, 125.0, 121.4, 116.4, 116.2, 66.6, 60.7, 60.7, 60.1, 59.6, 58.8, 55.6, 54.9, 41.9, 25.3, 24.7, 15.7, 8.9.

ESI-MS m/z: Calcd. for $C_{26}H_{28}N_4O_4$: 460.5. Found $(M+H)^+$: 461.1

Example 35



To a solution of **3** (2.38 g, 3.47 mmol) in dioxane (5 ml) 5.3M HCl in dioxane (34 ml) was added and the reaction was stirred at 23 °C for 45 minutes. Then Ac_2O (51 ml, 539.5 mmol) was added and the mixture was stirred for 4h. The reaction was cooled at 0 °C and partitioned between aqueous saturated Na_2CO_3 (300 ml) and ethyl acetate (300 ml) at this temperature. The organic phase was dried over sodium sulphate, filtered and concentrated. The residue was purified by flash column chromatography (SiO_2, gradient CH_2Cl_2 to CH_2Cl_2:ethyl acetate 1:2) to give **5** (1.75 g, 97%) as a yellow solid.

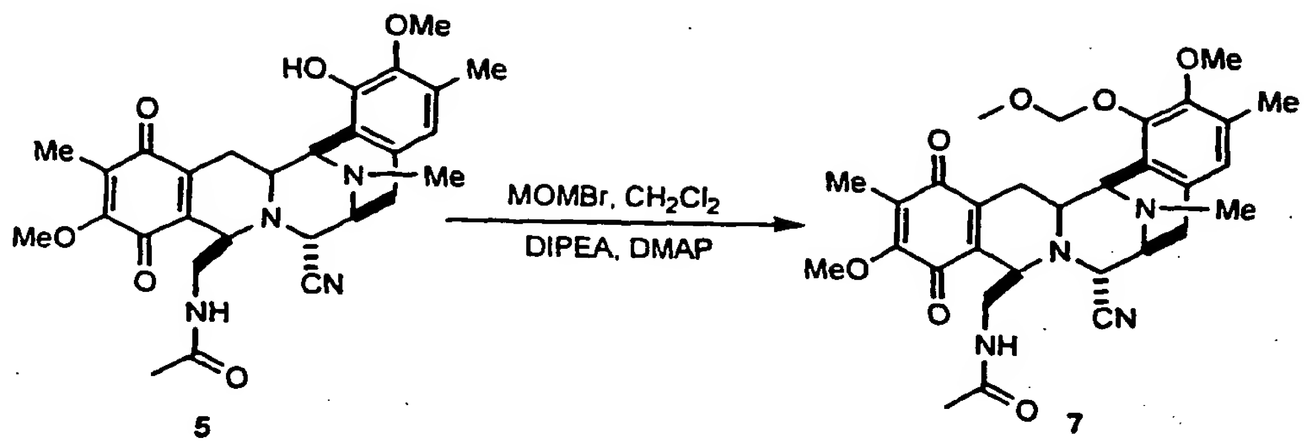
Rf: 0.53 (ACN:H_2O 3:2, RP-C18);

^1H NMR (300 MHz, $CDCl_3$): δ 6.51 (s, 1H), 5.98 (bs, 1H), 4.84 (dd, 1H), 4.17 (d, 1H), 4.00 (d, 1H), 3.99 (s, 3H), 3.85 (bs, 1H), 3.81 (m, 1H), 3.74 (s, 3H), 3.70 (d, 1H), 3.23 (m, 1H), 3.11 (dd, 1H), 3.09 (m, 1H), 2.93 (m, 2 h), 2.44 (d, 1H), 3.67 (s, 3H), 2.25 (s, 3H), 1.70 (s, 3H), 1.60-1.50 (m, 2 h), 1.29 (s, 3H);

^{13}C NMR (75 MHz, $CDCl_3$): δ 185.9, 180.8, 169.9, 160.2, 156.2, 147.0, 143.1, 140.4, 136.1, 130.6, 129.6, 127.9, 120.4, 117.2, 61.0, 60.7, 58.6, 56.1, 55.7, 55.1, 54.3, 41.8, 41.1, 25.7, 23.9, 22.2, 15.7, 8.7.

ESI-MS m/z: Calcd. for $C_{28}H_{32}N_4O_6$: 520.6. Found $(M+H)^+$: 521.1

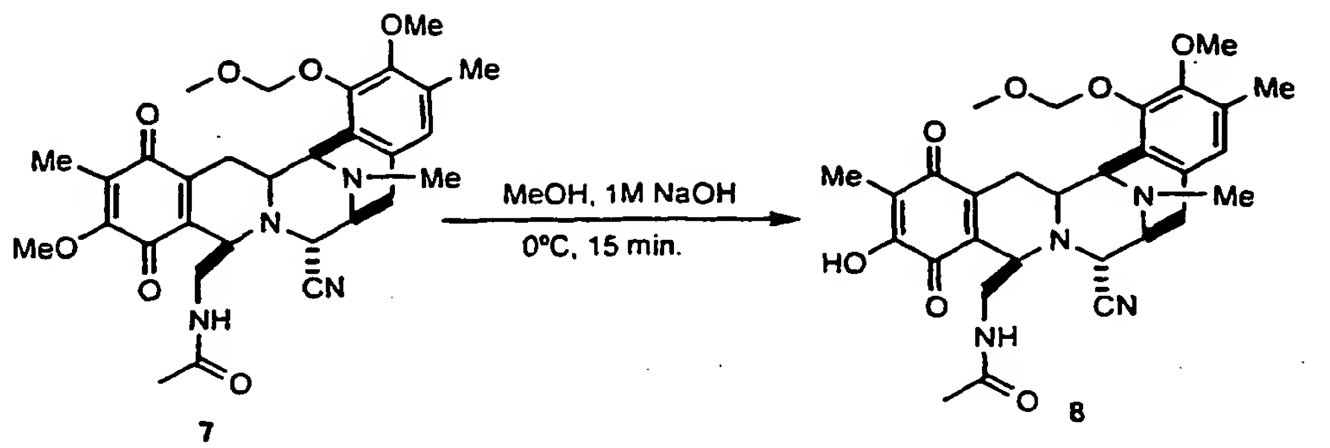
Example 36



To a solution of **5** (1.75 g, 3.36 mmol) in CH_2Cl_2 (17 ml) diisopropylethylamine (11.71 ml, 67.23 mmol), DMAP (20 mg, 0.17 mmol) and bromomethyl methyl ether (4.11 ml, 50.42 mmol) were added at 0 °C. After 6 h at 23 °C the reaction was partitioned between CH_2Cl_2 (50 ml) and aqueous saturated sodium bicarbonate (25 ml). The organic layer was dried over sodium sulphate and the solvent was eliminated under reduced pressure. The crude was purified by flash column chromatography (RP-18, CH_3CN/H_2O 1/1) to give **7** (1.32 g, 70%) as a yellow solid.

Rf: 0.34 (ACN:H_2O 2:3, RP-C18);
^1H NMR (300 MHz, $CDCl_3$): δ 6.74 (s, 1H), 5.14 (s, 2 h), 4.82 (m, 1H), 4.22 (d, 1H), 4.00 (s, 3H), 4.0 (m, 1H), 3.83 (m, 2 h), 3.7 (s, 3H), 3.58 (s, 3H), 3.4 (m, 1H), 3.2-2.95 (m, 6H), 2.43 (d, 1H), 2.37 (s, 3H), 2.22 (s, 3H), 1.89 (s, 3H), 1.5-1.4 (m, 2 h), 1.31 (s, 3H);
^{13}C NMR (75 MHz, $CDCl_3$): δ 185.9, 180.7, 169.6, 156.2, 148.9, 148.5, 140.3, 136.2, 131.3, 130.1, 127.7, 124.6, 123.7, 117.3, 99.5, 99.2, 60.9, 59.7, 58.8, 57.7, 56.4, 55.7, 55.0, 54.2, 51.0, 41.6, 41.0, 40.5, 25.5, 23.9, 22.3, 19.3, 15.6, 14.6, 8.6.
ESI-MS m/z: Calcd. for $C_{30}H_{36}N_4O_7$: 564.6. Found $(M+H)^+$: 565.3

Example 37



To a solution of **7** (0.37 g, 0.65 mmol) in methanol (74 ml) at 0 °C was added 1M sodium hydroxide (130 ml). The reaction was stirred for 15 minutes and then, quenched at 0 °C with 6M HCl to pH = 5. The mixture was extracted with ethyl acetate (3 x 50 ml) and the combined organic layers were dried over sodium sulphate and concentrated *in vacuo*. The residue was purified by flash column chromatography (RP-C18 CH_3CN:H_2O 1/:1) to afford **8** (232 mg, 65%) as a yellow oil.

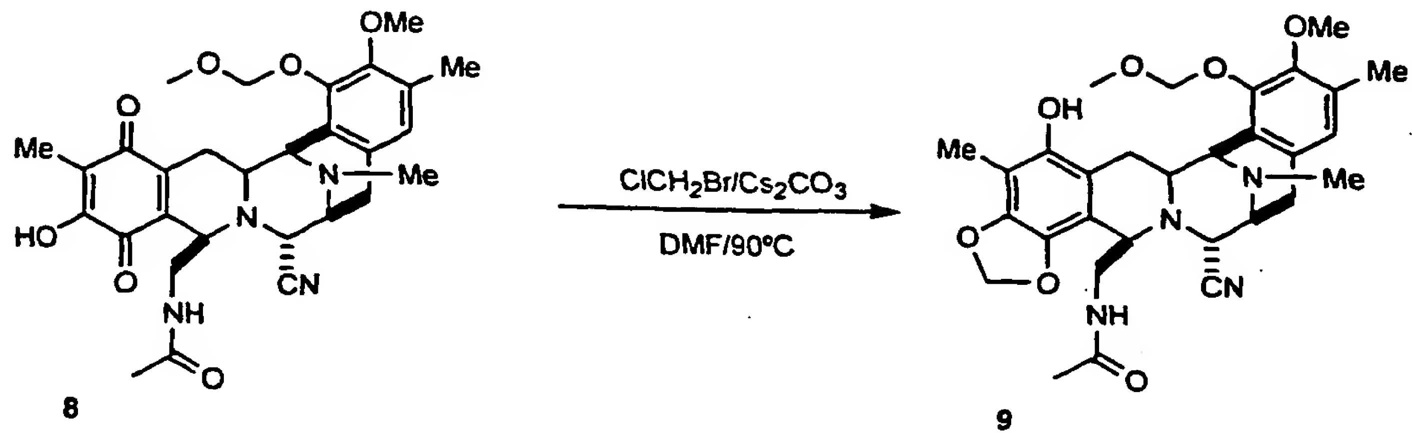
Rf: 0.5 (ACN:H_2O 3:2, RP-C18);

^{1}H NMR (300 MHz, $CDCl_3$): δ 6.75 (s, 1H), 5.15 (s, 2 h), 4.86 (m, 1H), 4.26 (d, 1H),), 4.01 (d, 1H), 3.88-3.81 (m, 2 h), 3.70 (s, 3H), 3.58 (s, 3H), 3.39 (m, 1H), 3.27-3.21 (m, 1H), 3.18-3.08 (m, 2 h), 3.03-2.97 (m, 1H) 2.47 (d, 1H), 2.37 (s, 3H), 2. 22 (s, 3H), 1.90 (s, 3H), 1.57-1.46 (m, 2 h), 1.33 (s, 3H);

^{13}C NMR (75 MHz,□$CDCl_3$): δ 185.3, 180.6, 175.9, 170.1, 151.5, 148.9, 148.6, 143.3, 133.7, 131.5, 129.9, 124.7, 123.5, 117.1, 117.0, 99.2, 59.8, 58.7, 57.8, 56.3, 55.3, 54.9, 54.3, 41.5, 40.7, 29.6, 25.5, 24.4, 22.2, 20.7, 15.7, 8.0.

ESI-MS m/z: Calcd. for $C_{29}H_{34}N_4O_7$: 550.6. Found $(M+H)^+$: 551.2

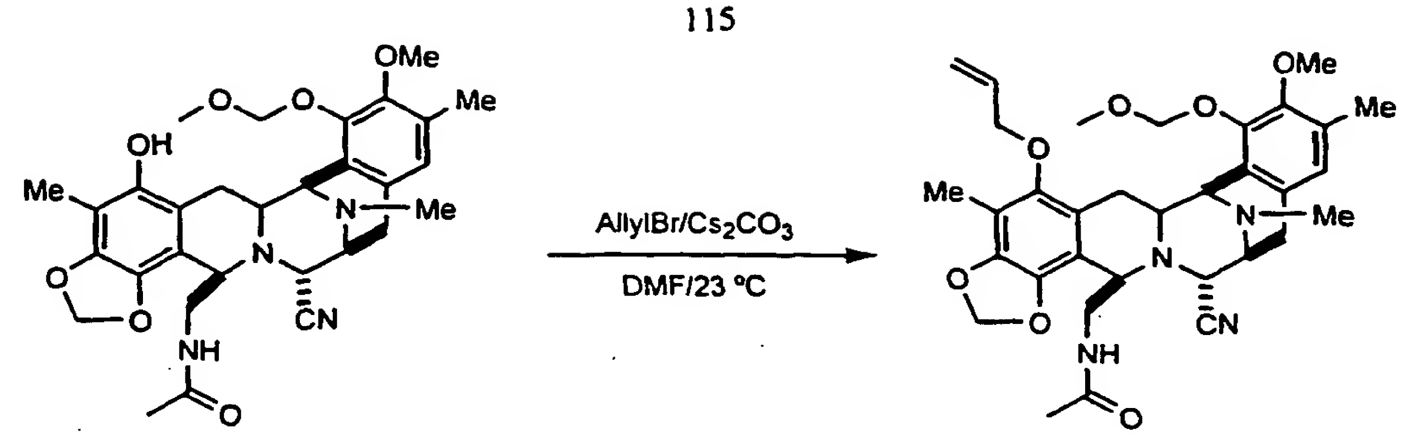
Example 38



To a degassed solution of compound **8** (240mg, 0.435 mmol) in DMF (30 ml) 10 % Pd/C (48 mg) was added and the reaction was stirred under H_2 (atmospheric pressure.) for 1h. The reaction was filtered through a pad of celite under Argon to a Schlenk tube, as a colourless solution, containing anhydrous Cs_2CO_3 (240 mg, 0.739 mmol). Then, bromochloromethane (0.566 ml, 8.71 mmol) was added. The tube was sealed and stirred at 90 °C for 3h. The reaction was cooled and filtrated through celite and washed with CH_2Cl_2. The organic layer was concentrated and dried (sodium sulphate) to afford **9** as a brown oil that was used in the next step with no further purification.

Rf: 0.36 (SiO_2, hexane:ethyl acetate 1:5)
^{1}H NMR (300 MHz, $CDCl_3$): δ 6.71 (s, 3H), 5.89 (d, 1H), 5.81 (d, 1H), 5.63 (bs, 1H), 5.33 (d, 1H), 5.17 (d, 1H), 4.97 (m, 1H), 4.20 (d, 1H), 4.09 (m, 1H), 3.99 (m, 1H), 3.68 (m, 1H), 3.65 (s, 6H), 3.59-3.47 (m, 4H), 3.37-3.27 (m, 2 h), 3.14- 2.97 (m, 2 h), 2.62 (d, 1H), 2.32 (s, 3H), 2.20 (s, 3H), 2.08 (s, 3H), 1.72 (m, 1H), 1.36 (s, 3H);
^{13}C NMR (75 MHz, $CDCl_3$): δ 169.8, 149.1, 147.4, 145.5, 136.2, 130.9, 130.8, 125.0, 122.9, 117.7, 112.6, 111.8, 106.4, 100.8, 99.8, 59.8, 58.9, 57.7, 56.6, 56.4, 55.5, 55.2, 41.6, 40.1, 29.6, 25.9, 25.0, 22.6, 15.6, 8.8.
ESI-MS m/z: Calcd. for $C_{30}H_{36}SiN_4O_7$: 564.6. Found $(M+H)^+$: 565.3.

Example 39

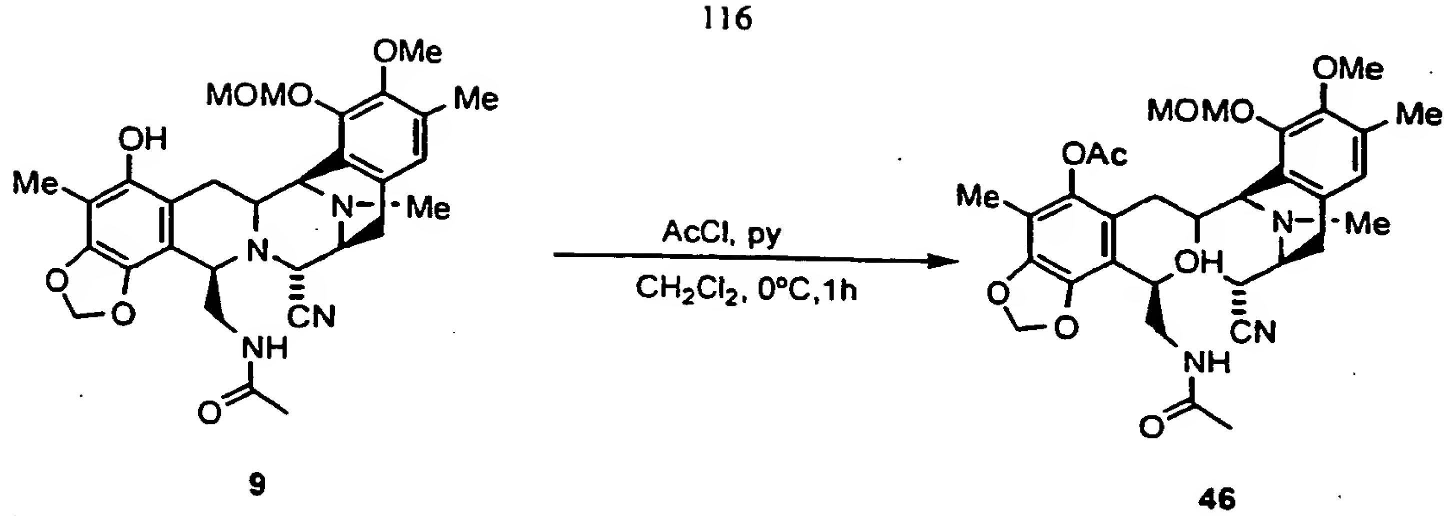


9 10

To a flask containing **9** (245 mg, 0.435 mmol) in DMF, (4 ml), cesium carbonate (425 mg, 1.30 mmol) and allyl bromide (376 ml, 4.35 mmol) were added at 0 °C and the mixture was stirred at 23 °C for 1h. The reaction was filtered though a pad of celite and partitioned between CH_2Cl_2 (25 ml) and H_2O (10 ml). The organic phase was dried (sodium sulphate) and concentrated at reduced pressure to afford a residue that was purified by flash column chromatography (SiO_2, $CHCl_3$:ethyl acetate 1:2) to give **10** as a yellow oil. (113 mg, 43 %).

Rf: 0.36 (hexane:ethyl acetate 1:5)
^{1}H NMR (300 MHz, $CDCl_3$): δ 6.74 (s, 1H), 6.3-6.0 (m, 1H), 5.94 (d, 1H), 5.87 (d, 1H), 5.43-5.36 (m, 2 h), 5.22 (s, 2 h), 5.00 (m, 1H), 4.22 (m, 1H), 4.17-4.01 (m, 1H), 3.98 (m, 2 h), 3.71-3.67 (m, 1H), 3.69 (s, 3H), 3.62-3.51 (m, 3H), 3.58 (s, 3H), 3.39-3.37 (m, 1H), 3.31-3.26 (m, 3H), 3.09 (dd, 1H), 2.56 (d, 1H), 2.36 (s, 3H), 2.21 (s, 3H), 2.11 (s, 3H), 2.24-2.10 (m, 1H), 1.82-1.73 (m, 1H), 1.24 (bs, 3H)
^{13}C NMR (75 MHz, $CDCl_3$): δ 169.4, 148.8, 148.3, 139.1, 133.7, 130.9, 130.3, 125.2, 120.2, 117.7, 113.1, 112.6, 101.3, 99.3, 74.1, 59.7, 59.3, 57.8, 57.0, 56.1, 56.1, 55.2, 41.6, 41.0, 40.9, 29.7, 26.3, 22.5, 15.6, 9.3
ESI-MS m/z: Calcd. for $C_{33}H_{40}N_4O_7$: 604.7. Found $(M+H)^+$: 605.3.

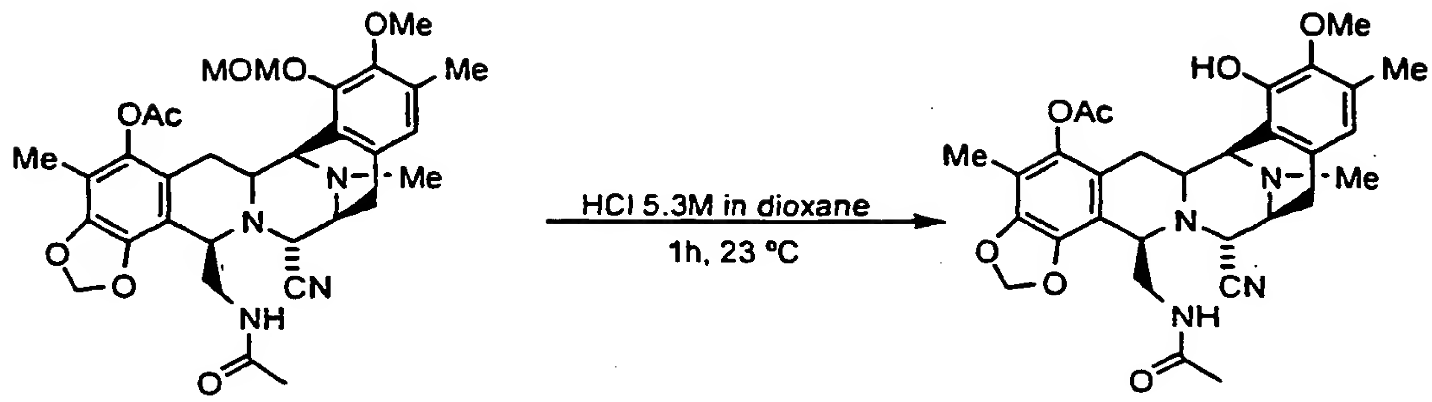
Example 40



To a solution of **9** (22 mg, 0.039 mmol) in CH_2Cl_2 (0.2 ml), acetyl chloride (2.79 ml, 0.039 mmol) and pyridine (3.2 ml, 0.039 mmol) were added at 0 °C. The reaction mixture was stirred for 1h and then, the solution was diluted with CH_2Cl_2 (10 ml) and washed with 0.1 N HCl (5 ml). The organic layer was dried over sodium sulphate, filtered, and the solvent was eliminated under reduced pressure to afford **46** (22 mg, 93%) as a white solid.

Rf: 0.4 (hexane:ethyl acetate 1:5).
^1H NMR (300 MHz, $CDCl_3$). δ 6.74 (s, 1H), 5.97 (d, J= 0.9 Hz, 1H), 5.91 (d, J= 0.9 Hz, 1H), 5.12 (d, J= 5.7 Hz, 2 h), 5.04 (d, J= 5.7 Hz, 1H) 4.90 (t, J= 6 Hz, 1H), 4.17 (d, J= 2.7 Hz, 1H), 4.05 (d, J= 2.7 Hz, 1H), 4.01 (bs, 1H), 3.71 (s, 3H), 3.57 (s, 3H), 3.50-3.44 (m, 2 h), 3.38-3.36 (m, 1H), 3.30-3.26 (m, 1H), 3.00 (dd, J_1= 7.8 Hz, J_2= 18.0 Hz, 1H), 2.79 (d, J= 12.9 Hz, 1H), 2.60 (d, J=18.0 Hz, 1H), 2.35 (s, 3H), 2.32 (s, 3H), 2.21 (s, 3H), 2.00 (s, 3H), 1.68 (dd, J_1=11.7 Hz, J_2= 15.6 Hz, 1H).
ESI-MS m/z: Calcd. for $C_{32}h_{38}N_4O_8$: 606.67. Found $(M+H)^+$: 607.3.

Example 41

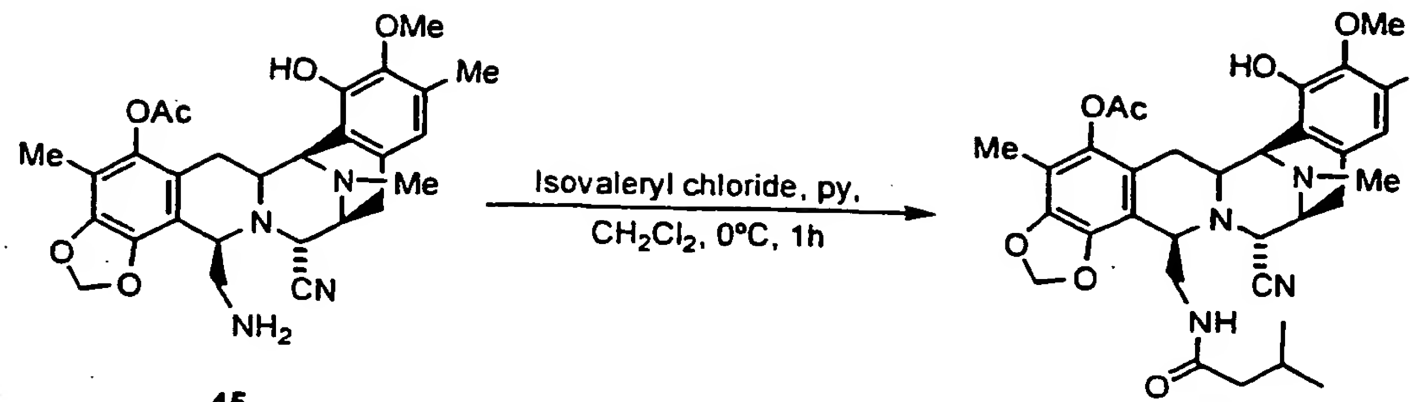


46 47

To a solution of **46** (8 mg, 0.013 mmol) in dioxane (0.1 ml), 5.3N HCl/dioxane (0.5 ml) was added and the reaction was stirred at 23 °C for 1h. Then, the solution was diluted with CH_2Cl_2 (5 ml) and washed with 0.1 N HCl (3 ml). The organic layer was dried over sodium sulphate, filtered, and the solvent was eliminated under reduced pressure to afford **47** (5 mg, 70%) as a white solid.

Rf: 0.4 (hexane:ethyl acetate 1:5).
^{1}H NMR (300 MHz, $CDCl_3$). δ 6.51 (s, 1H), 5.97 (d, *J*= 1.2 Hz, 1H), 5.91 (d, *J*= 1.2 Hz, 1H), 4.97 (bs, 1H), 4.11 (bs, 1H), 4.04-4.02 (m, 2 h), 3.75 (s, 3H),), 3.65 (d, *J*= 2.1 Hz, 2 h), 3.56-3.30 (m, 2 h), 3.04 (dd, J_1= 7.5 Hz, J_2= 18 Hz, 1H), 2.80 (d, *J*= 14.4 Hz, 1H), 2.59 (d, *J*= 18.3 Hz, 1H), 2.33 (s, 3H), 2.24 (s, 3H), 2.00 (s, 3H), 1.76 (dd, J_1= 12.0 Hz, J_2= 15.9 Hz, 1H), 1.33 (s, 3H), 1.25 (s, 3H).
ESI-MS m/z: Calcd. for $C_{30}H_{34}N_4O_7$: 562.61. Found $(M+H)^+$: 563.3.

Example 42



45

48

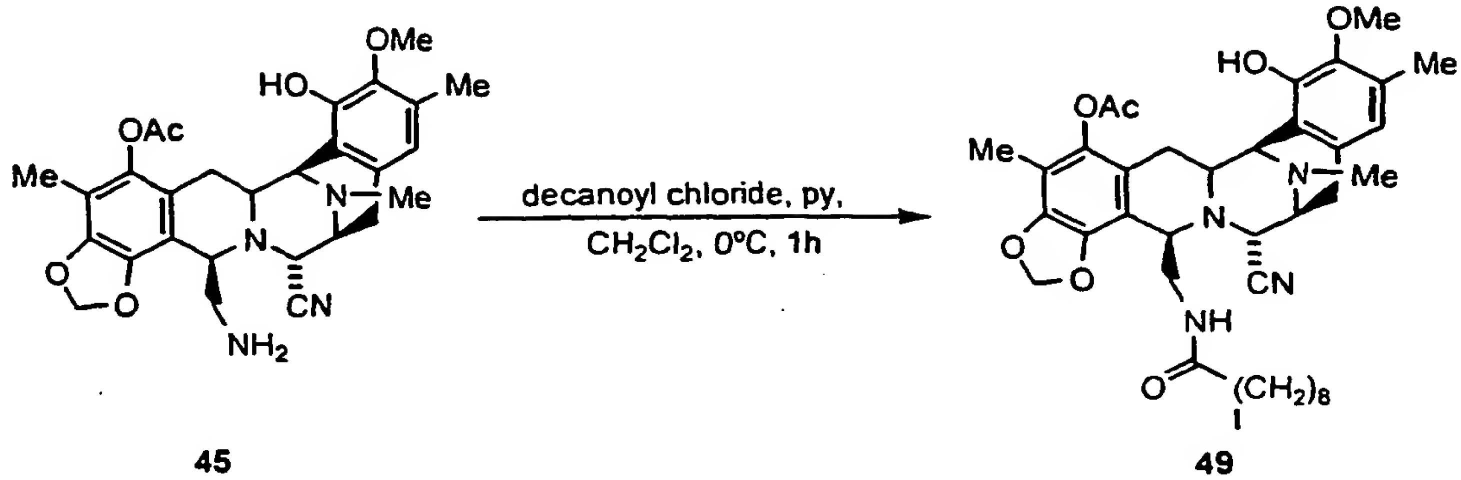
To a solution of **45** (10 mg, 0.0192 mmol) in CH_2Cl_2 (0.3 ml), isovaleryl chloride (2.34 ml, 0.0192 mmol) and pyridine (1.55 ml, 0.0192 mmol) were added at 0 °C. The reaction mixture was stirred for 1h and then, the solution was diluted with CH_2Cl_2 (5 ml) and washed with 0.1 N HCl (3 ml). The organic layer was dried over sodium sulphate, filtered, and the solvent was eliminated under reduced pressure. The residue was purified by flash column chromatography (SiO_2, Hex: ethyl acetate 1:2) to afford **48** (11 mg, 95%) as a white solid.

Rf: 0.12 (Hex: ethyl acetate 1:2).

^1H NMR (300 MHz, $CDCl_3$): δ 6.50 (s, 1H), 5.98 (d, J= 1.5Hz, 1H), 5.91(d, J= 1.5 Hz, 1H), 5.75 (s, 1H), 5.02 (t, J= 5.4 Hz, 1H), 4.10 (d, J= 1.5 Hz, 1H), 4.06 (d, J= 2.7 Hz, 1H), 4.02 (d, J= 2.7 Hz, 1H), 3.77 (s, 3H), 3.76-3.71 (m, 1H), 3.86-3.28 (m, 3H), 3.04 (dd, J_1= 8.1 Hz, J_2= 18.3Hz, 1H), 2.78 (d, J=15.9 Hz, 1H), 2.55 (d, J=18 Hz, 1H), 2.32 (s, 6H), 2.26 (s, 3H), 1.98 (s, 3H), 1.84-1.68 (m, 2 h), 1.36 (d, J= 7.2 Hz, 2 h), 0.69 (d, J= 6.6 Hz, 3H), 0.62 (d, J=6.6 Hz, 3H).

ESI-MS m/z: Calcd. for $C_{33}H_{40}N_4O_7$: 604.69. Found $(M+H)^+$: 605.3.

Example 43



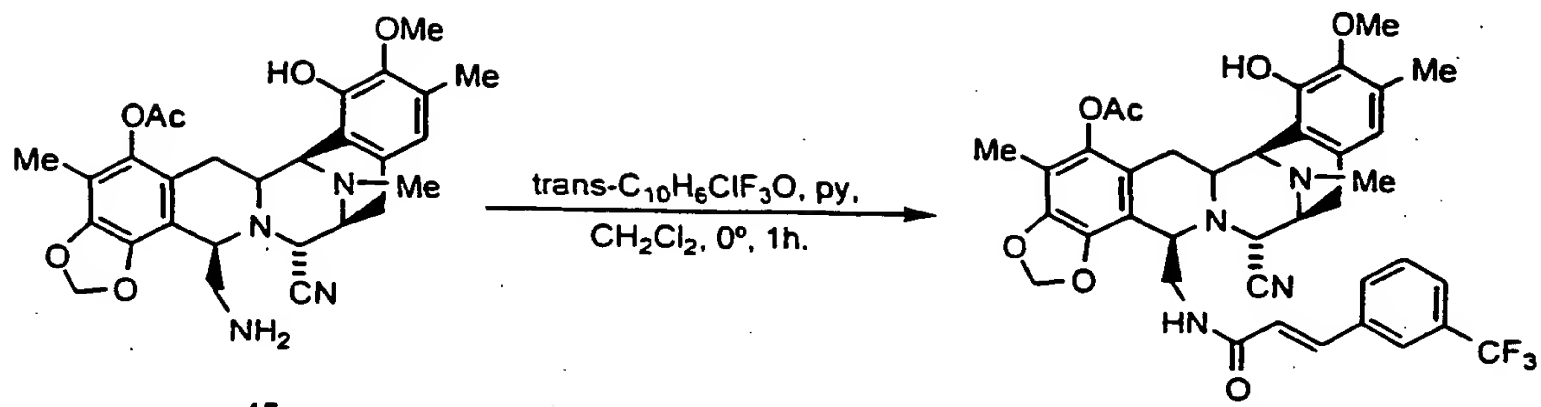
To a solution of **45** (10 mg, 0.0192 mmol) in CH_2Cl_2 (0.3 ml), isovaleryl chloride (3.98 ml, 0.0192 mmol) and pyridine (1.55 ml, 0.0192 mmol) were added at 0 °C. The reaction mixture was stirred for 1h and then, the solution was diluted with CH_2Cl_2 (5 ml) and washed with 0.1 N HCl (3 ml). The organic layer was dried over sodium sulphate, filtered, and the solvent was eliminated under reduced pressure. The residue was purified by flash column chromatography (SiO_2, Hex: ethyl acetate 1:2) to afford **49** (12.4 mg, 96%) as a white solid.

Rf: 0.7 (ethyl acetate:methanol10:1).

^{1}H NMR (300 MHz, $CDCl_3$): δ 6.50 (s, 1H), 5.98 (d, J= 1.5Hz, 1H), 5.91 (d, J= 1.5 Hz, 1H), 5.73 (s, 1H), 5.08 (t, J= 5.4 Hz, 1H), 4.10 (d, J= 1.5 Hz, 1H), 4.05 (m., 1H), 4.01 (m, 1H), 3.76 (s, 3H), 3.65-3.61 (m, 1H), 3.40-3.27 (m, 3H), 3.03 (dd, J_1= 8.1 Hz, J_2= 18.6 Hz, 1H), 2.78 (d, J=13.2 Hz, 1H), 2.57 (d, J=18.3 Hz, 1H), 2.32 (s, 3H), 2.31 (s, 3H), 2.25 (s, 3H), 1.99 (s, 3H), 1.79 (dd, J_1= 12.0 Hz, J_2= 16.5 Hz, 1H), 1.73-1.42 (m, 4H), 1.33-1.18 (m, 10H), 1.03 (m, 2 h), 0.87 (t, J= 6.6 Hz, 3H).

ESI-MS m/z: Calcd. for $C_{38}H_{50}N_4O_7$: 674.83. Found $(M+H)^+$: 675.5.

Example 44



45 50

To a solution of **45** (14.5 mg, 0.0278 mmol) in CH_2Cl_2 (0.3 ml), trans-3-trifluoromethyl cinnamoyl chloride (4.76 ml, 0.0278 mmol) and pyridine (2.25 ml, 0.0278 mmol) were added at 0 °C. The reaction mixture was stirred for 1h and then, the solution was diluted with CH_2Cl_2 (5 ml) and washed with 0.1 N HCl (3 ml). The organic layer was dried over sodium sulphate, filtered, and the solvent was eliminated under reduced pressure. The residue was purified by flash column chromatography (SiO_2, Hex: ethyl acetate 1:1) to afford **50** (18.7 mg, 94%) as a white solid.

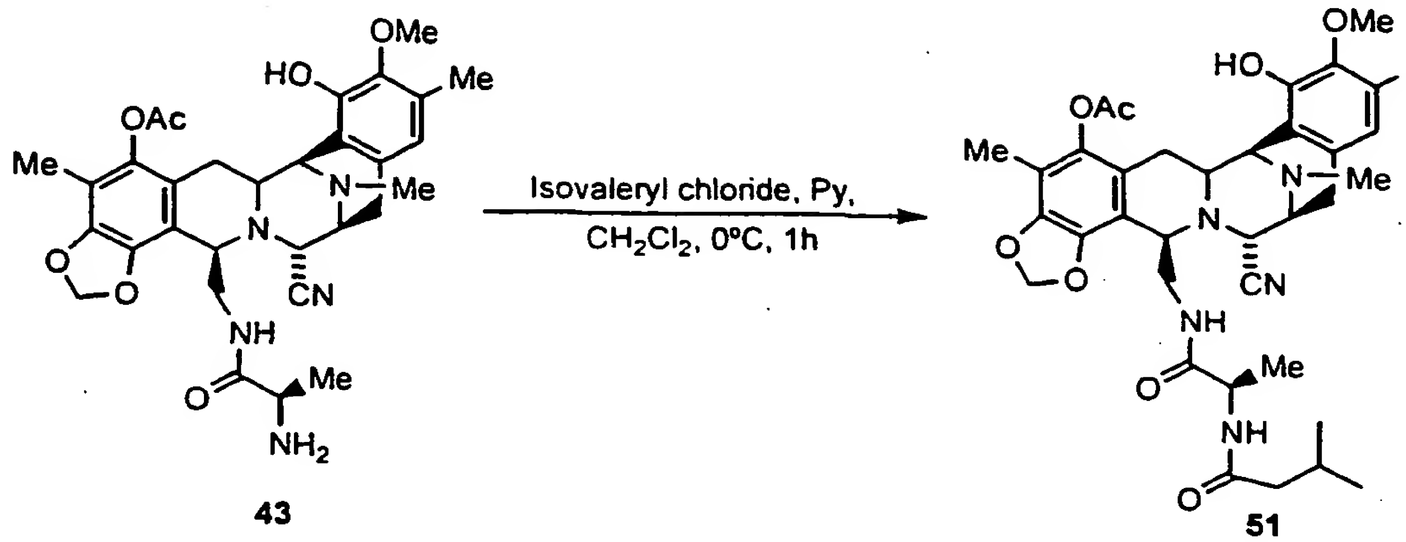
Rf: 0.64 (ethyl acetate:methanol5:1).

^1H NMR (300 MHz, CH_3OD). δ 7.74-7.55 (m, 4H), 7.23 (d, J= 16.0 Hz, 1H), 6.34 (s, 1H), 6.12 (d, J= 16.0 Hz, 1H), 6.07 (d, J= 0.9 Hz, 1H), 5.96 (d, J= 0.9 Hz, 1H), 4.39 (d, J= 2.4 Hz, 1H), 4.07-4.05 (m, 1 H), 3.81 (bs, 1H), 3.46-3.51 (m, 3H), 3.42 (s, 3H), 3.09 (br d, J= 12.0 Hz, 1H), 2.94-2.85 (m, 2 h), 2.74 (d, J=18.3 Hz, 1H), 2.38 (s, 3H), 2.23 (s, 3H), 2.02 (s, 3H), 1.80 (s, 3H), 1.84-1.75 (m, 1H).

^{13}C NMR (75 MHz, $CDCl_3$)): δ 168.7, 165.3, 146.5, 144.7, 142.6, 140.6, 138.0, 135.9, 131.0, 130.9, 129.1, 128.6, 125.8, 125.7, 124.5, 124.4, 122.7, 121.2, 117.8, 116.5, 113.0, 112.0, 101.7, 60.4, 59.1, 56.5, 56.4, 55.6, 55.3, 41.8, 40.3, 26.6, 25.1, 20.3, 15.4, 9.3.

ESI-MS m/z: Calcd. for $C_{38}H_{37}F_3N_4O_7$: 718.72. Found $(M+H)^+$: 719.3.

Example 45



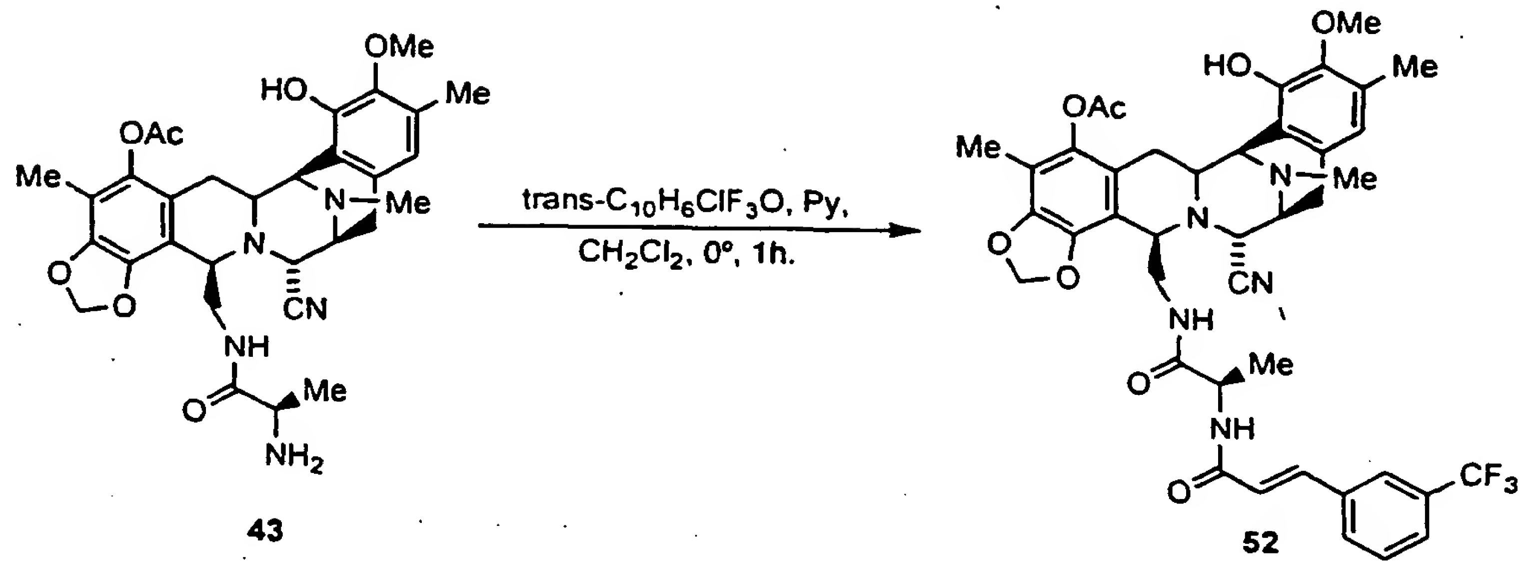
To a solution of **43** (33 mg, 0.0557 mmol) in CH_2Cl_2 (0.4 ml), isovaleryl chloride (6.79 ml, 0.0557 mmol) and pyridine (4.5 ml, 0.0557 mmol) were added at 0 °C. The reaction mixture was stirred for 1h and then, the solution was diluted with CH_2Cl_2 (5 ml) and washed with 0.1 N HCl (3 ml). The organic layer was dried over sodium sulphate, filtered, and the solvent was eliminated under reduced pressure. The residue was purified by flash column chromatography (SiO_2, Hex: ethyl acetate 1:2) to afford **51** (34 mg, 91%) as a white solid.

Rf: 0.09 (Hex: ethyl acetate 1:2).

^1H NMR (300 MHz, $CDCl_3$): δ 6.46 (s,1H), 6.10 (bs, 1H), 5.99 (d, J= 0.9Hz, 1H), 5.90 (d, J= 0.9 Hz, 1H), 5.30 (t, J= 6.0 Hz, 1H), 4.10-4.05 (m, 3H),3.81 (bs, 1H), 3.74 (s, 3H), 3.54 (bs,1H), 3.38-3.36 (m, 1H), 3.29-3.21 (m, 1H), 3.00 (dd, J_1= 8.0 Hz, J_2= 18.0 Hz, 1H), 2.25 (s, 3H), 2.20 (s, 3H), 2.00 (s, 3H), 1.95-1.90 (m, 3H), 0.87 (d, J=6.6 Hz, 6H), 0.76 (d, J=6.0 Hz, 3H).

ESI-MS m/z: Calcd. for $C_{36}H_{45}N_5O_8$: 675.77. Found $(M+H)^+$: 676.3.

Example 46



43 52

To a solution of **43** (33 mg, 0.0557 mmol) in CH_2Cl_2 (0.4 ml), trans-3-trifluoromethyl cinnamoyl chloride (9.52 ml, 0.0557 mmol) and pyridine (4.5 ml, 0.0557 mmol) were added at 0 °C. The reaction mixture was stirred for 1h and then, the solution was diluted with CH_2Cl_2 (5 ml) and washed with 0.1 N HCl (3 ml). The organic layer was dried over sodium sulphate, filtered, and the solvent was eliminated under reduced pressure. The residue was purified by flash column chromatography (SiO_2, Hex: ethyl acetate 1:2) to afford **52** (40 mg, 92%) as a white solid.

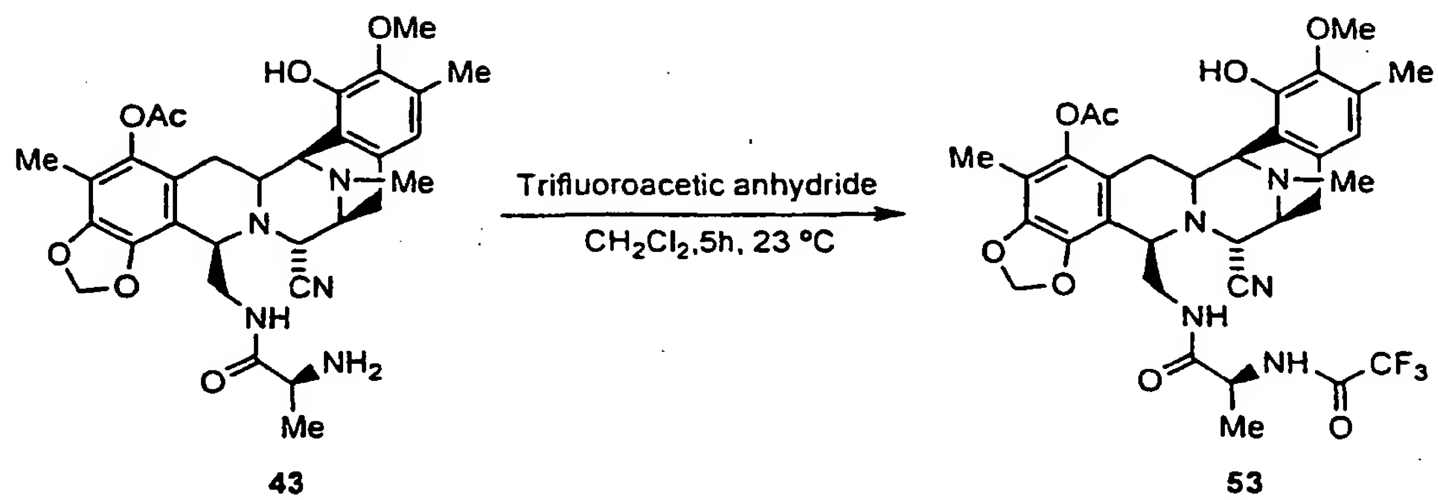
Rf: 0.21 (hexane:ethyl acetate 1:2).

^{1}H NMR (300 MHz, CD_3OD). δ 7.74-7.47 (m, 4H), 6.49 (s, 1H), 6.40 (d, J= 15.6 Hz, 1H), 6.00 (d, J= 1.5 Hz, 1H), 5.90 (d, J= 1.5 Hz, 1H), 5.47 (t, J= 6 Hz, 1H), 4.12-4.09 (m, 3H), 3.93 (bs, 1H), 3.71 (s, 3H), 3.59-3.58 (m, 1H), 3.38 (d, J=7.8 Hz, 1H), 3.29 (d, J=12.0 Hz, 1H), 3.00 (dd, J_1= 8.1 Hz, J_2= 18.3 Hz, 1H), 2.79-2.78 (m, 1H), 2.65 (d, J=18.3 Hz, 1H) 2.29 (s, 6H), 2.28 (s, 3H), 2.22 (s, 3H), 1.84-1.80 (m, 1H), 0.85-0.84 (m, 3H).

^{13}C NMR (75 MHz, $CDCl_3$) δ 171.9, 168.8, 164.4, 146.9, 144.6, 143.0, 140.5, 140.5, 139.3, 135.7, 131.1, 131.0, 129.4, 129.1, 126.0, 124.1, 124.0, 122.4, 121.1, 120.7, 120.6, 117.7, 116.9, 112.8, 112.0, 101.6, 60.6, 59.3, 57.1, 56.3, 55.9, 55.2, 49.0, 41.7, 49.9, 26.5, 25.1, 20.2, 18.4, 15.7, 9.3.

ESI-MS m/z: Calcd. for $C_{41}H_{42}F_3N_5O_8$: 789.8. Found $(M+H)^+$: 790.3.

Example 47



To a solution of **43** (10 mg, 0.0169 mmol) in CH_2Cl_2 (0.2 ml) trifluoroacetic anhydride (2.38μl, 0.0169 mmol) was added at 23 °C. The reaction mixture was stirred for 5h and then, the solution was diluted with CH_2Cl_2 (5 ml) and washed with 0.1 N HCl (3 ml). The organic layer was dried over sodium sulphate, filtered, and the solvent was eliminated under reduced pressure. The residue was purified by flash column chromatography (SiO_2, Hex: ethyl acetate 3:2) to afford **53** (10.7 mg, 93%) as a white solid.

Rf: 0.57 (ethyl acetate:methanol5:1).

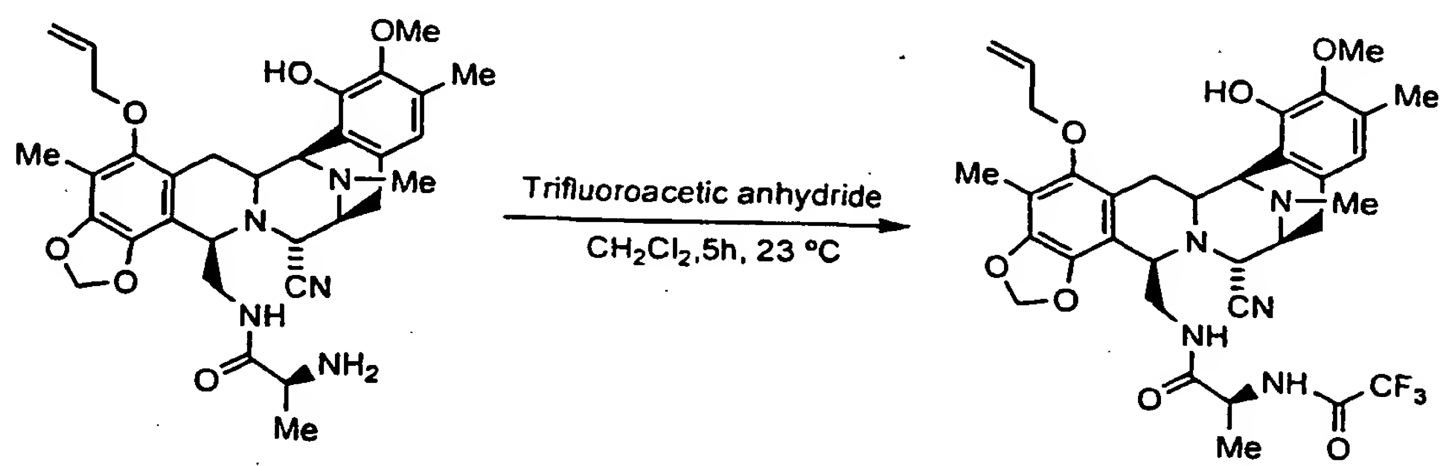
^1H NMR (300 MHz, $CDCl_3$) δ 6.45 (s, 1H), 6.00 (d, J= 1.2 Hz, 1H), 5.90 (d, J= 1.2 Hz, 1H), 5.87 (bs, 1H), 5.32 (bs, 1H), 4.12(d, J= 2.1 Hz, 1H), 4.08 (d, J= 1.8 Hz, 1H), 3.78-3.56 (m, 3H), 3.72 (s, 3H), 3.40 (d, J= 8.1 Hz, 1H), 3.25 (d, J= 9.3 Hz, 1H), 3.00 (dd, J_1= 8.4 Hz, J_2= 18.0 Hz, 1H), 2.77 (dd, J_1= 2.1 Hz, J_2= 15.9 Hz, 1H), 2.68 (d, J= 18.6 Hz, 1H), 2.30 (s, 3H), 2.28 (s, 3H), 2.22 (s, 3H), 2.00 (s, 3H), 1.75 (dd, J_1= 11.4 Hz, J_2= 15.9 Hz, 1H), 0.69 (d, J= 6.3 Hz, 3H).

^{13}C NMR (75 MHz, $CDCl_3$) δ 170.1, 168.6,156.0, 147.0, 144.6, 143.0, 140.6, 140.4, 131.0, 129.4, 120.9, 120.7, 117.6, 116.8, 112.4, 112.1,

101.6, 60.5, 59.0, 57.1, 56.3, 55.6, 55.2, 48.7, 41.6, 39.4, 26.5, 24.9, 20.2, 17.8, 15.4, 9.2.

ESI-MS m/z: Calcd. for $C_{33}H_{36}F_3N_5O_8$: 687.63. Found $(M+H)^+$: 688.66.

Example 48



19 54

To a solution of **19** (11 mg, 0.0169 mmol) in CH_2Cl_2 (0.2 ml) trifluoroacetic anhydride (2.38 ml, 0.0169 mmol) was added at 23 °C. The reaction mixture was stirred for 5h and then, the solution was diluted with CH_2Cl_2 (5 ml) and washed with 0.1 N HCl (3 ml). The organic layer was dried over sodium sulphate, filtered, and the solvent was eliminated under reduced pressure. The residue was purified by flash column chromatography (SiO_2, Hex: ethyl acetate 3:2) to afford **54** (10.7 mg, 93%) as a white solid.

Rf: 0.6 (ethyl acetate:methanol5:1).

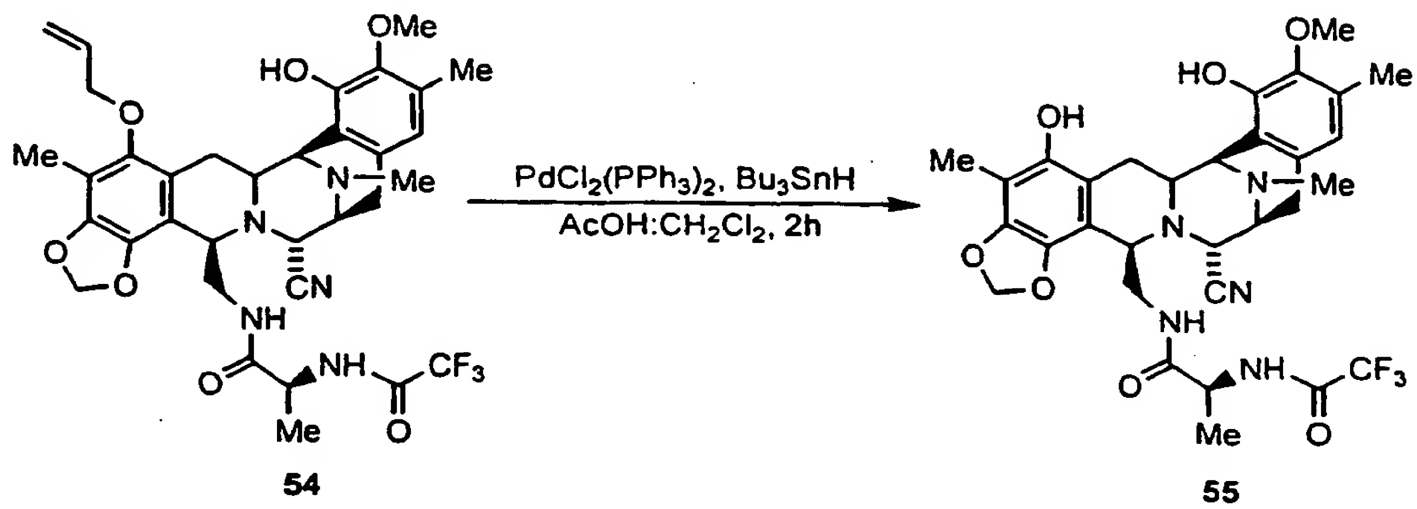
^1H NMR (300 MHz, $CDCl_3$) δ 7.33 (d, J= 6.3 Hz, 1H), 6.45 (s, 1H), 6.04 (m, 1H), 5.95 (d, J= 1.5 Hz, 1H), 5.84 (d, J= 1.5 Hz, 1H), 5.32 (m, 2 h), 5.21 (m, 1H), 4.11 (m, 4H), 3.73 (s, 3H), 3.64 (m, 2 h), 3.51 (m, 1H), 3.37 (d, J= 7.8 Hz, 1H), 3.22 (m, 2 h), 3.03 (dd, 1H, J_1= 8.1 Hz, J_2= 18.3 Hz, 1H), 2.60 (d, J= 18.3 Hz, 1H), 2.29 (s, 3H), 2.24 (s, 3H), 2.08 (s, 3H), 1.86 (dd, J_1= 12 Hz, J_2= 16.2 Hz, 1H), 0.82 (d, J= 7.2 Hz, 3H).

^{13}C NMR (75 MHz, $CDCl_3$) δ 170.0, 156.0, 148.4, 147.1, 144.3, 143.0, 138.7, 133.8, 130.5, 129.4, 120.6, 120.4, 117.6, 117.5, 117.0, 113.5,

112.5, 112.4, 101.1, 74.1, 66.8, 60.4, 59.3, 56.9, 56.6, 56.3, 55.4, 48.7, 41.6, 40.1, 26.2, 25.0, 17.6, 15.4, 9.1.

ESI-MS m/z: Calcd. for $C_{35}H_{39}F_3N_5O_7$: 685.69. Found $(M+H)^+$: 686.3.

Example 49



To a solution of **54** (100 mg, 0.415 mmol) in CH_2Cl_2 (4 ml), acetic acid (40 ml), $(PPh_3)_2PdCl_2$ (8.4 mg, 0.012 mmol) and Bu_3SnH (157 ml, 0.56 mmol) were added at 23 °C. After stirring at that temperature for 2 h the reaction was poured into a pad of flash column (SiO_2, gradient Hex to hexane:ethyl acetate 2:1) to afford **55** (90 mg, 96%) as a white solid.

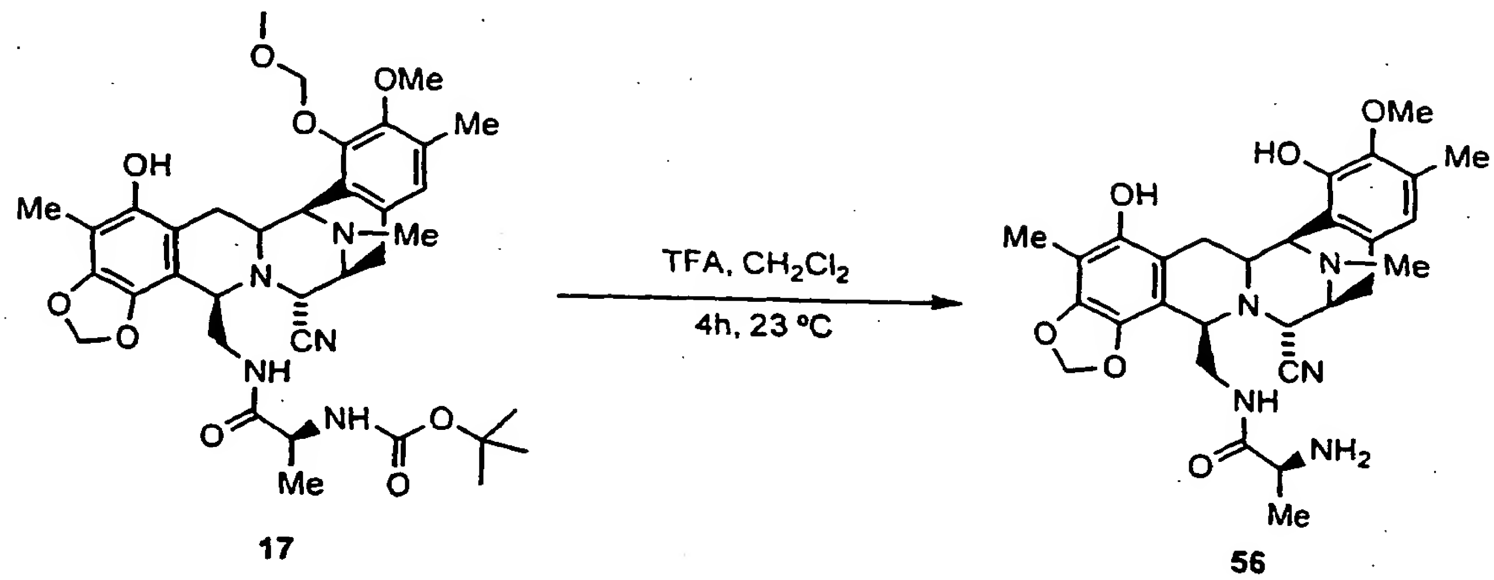
Rf: 0.6 (hexane:ethyl acetate 1:2).

^1H NMR (300 MHz, $CDCl_3$) δ 7.55 (d, J= 7.2 Hz, 1H), 6.45 (s, 1H), 5.90 (d, J= 1.2 Hz, 1H), 5.82 (d, J= 1.2 Hz, 1H), 5.37 (t, J= 6.0 Hz, 1H), 4.15 (d, J= 2.1 Hz, 1H), 4.04 (d, J= 1.8 Hz, 1H), 3.70 (s, 3H), 3.66-3.53 (m, 2 h), 3.37-3.31 (m, 2 h), 3.19-3.15 (d, J= 11.7 Hz, 1H), 3.08-3.00 (m, 2 h), 2.56 (d, J=18.3 Hz, 1H), 2.30 (s, 3H), 2.24 (s, 3H), 2.04 (s, 3H), 1.91 (dd, J_1= 12.0 Hz, J_2= 15.6 Hz, 1H), 0.84 (d, J= 6.9 Hz, 3H).

^{13}C NMR (75 MHz, $CDCl_3$) δ 170.1, 156.3, 147.3, 144.9, 144.4, 143.3, 136.7, 130.7, 129.3, 120.6, 117.6, 117.4, 114.4, 112.1, 107.7, 101.0, 85.8, 60.5, 59.3, 56.5, 56.4, 56.2, 55.2, 48.9, 41.6, 40.9, 25.7, 25.3, 18.0, 15.6, 8.7.

ESI-MS m/z: Calcd. for $C_{32}h_{35}F_3N_5O_7$: 645.63. Found $(M+H)^+$: 646.2.

Example 50



To a solution of **17** (200 mg, 0.288 mmol) in CH_2Cl_2 (1.44 ml), trifluoroacetic acid (888 ml, 11.53 mmol) was added and the reaction mixture was stirred for 4h at 23 °C. The reaction was quenched at 0 °C with saturated aqueous sodium bicarbonate (60 ml) and extracted with ethyl acetate (2 x 70 ml). The combined organic layers were dried (sodium sulphate) and concentrated *in vacuo* to afford **56** (147 mg, 93%) as a white solid that was used in subsequent reactions with no further purification.

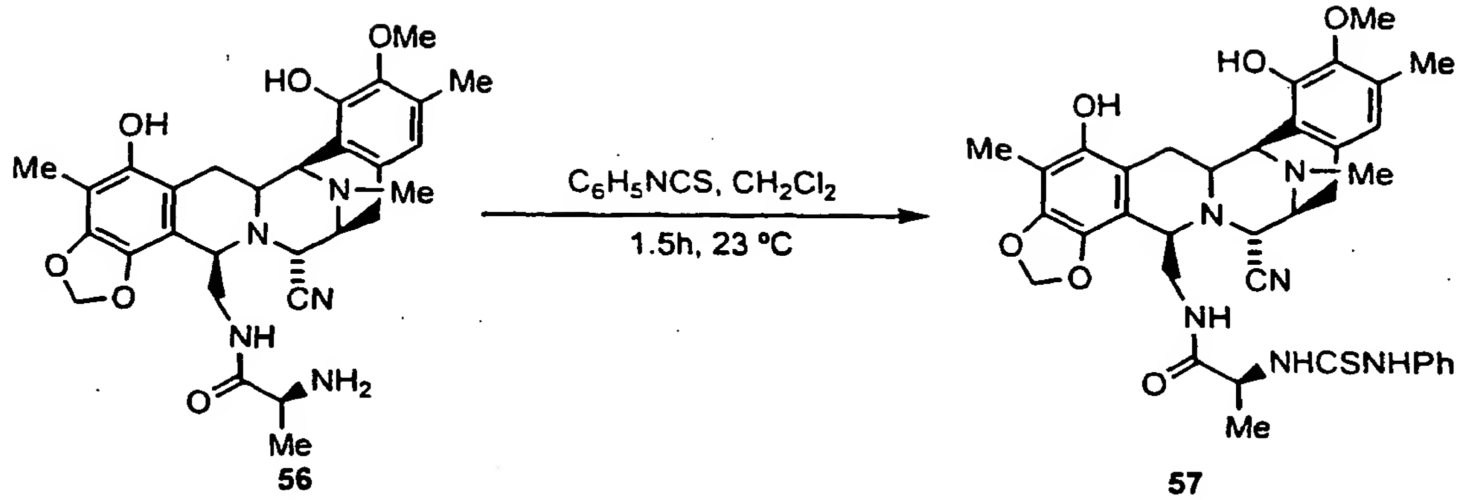
Rf: 0.19 (ethyl acetate:methanol5:1).

^1H NMR (300 MHz, CD_3OD). δ 6.48 (s, 1H), 5.88, d, J= 0.9 Hz, 1H), 5.81 (d, J= 0.9 Hz, 1H), 4.35 (d, J= 2.4 Hz, 1H),4.15 (d, J= 1.8 Hz, 1H), 3.99-3.98 (m, 1H), 3.70 (s, 3H), 3.52-2.96 (m, 7H), 2.68 (d, J= 18.3 Hz, 1H), 2.24 (s, 3H), 2.23 (s, 3H), 2.06 (s, 3H), 1.85 (dd, J_1= 11.7 Hz, J_2= 15.6 Hz, 1H), 0.91 (d, J= 6.6 Hz, 3H).

^{13}C NMR (75 MHz, CD_3OD): δ 173.2, 149.1, 145.6, 144.9, 138.0, 132.2, 130.6, 121.4, 119.6, 117.4, 114.3, 109.2, 102.5, 82.3, 60.4, 58.4, 58.3, 57.8, 56.6, 50.1, 42.3, 41.6, 27.8, 26.2, 19.5, 15.5, 9.8.

ESI-MS m/z: Calcd. for $C_{29}H_{35}N_5O_6$: 549.62. Found $(M+H)^+$: 550.3.

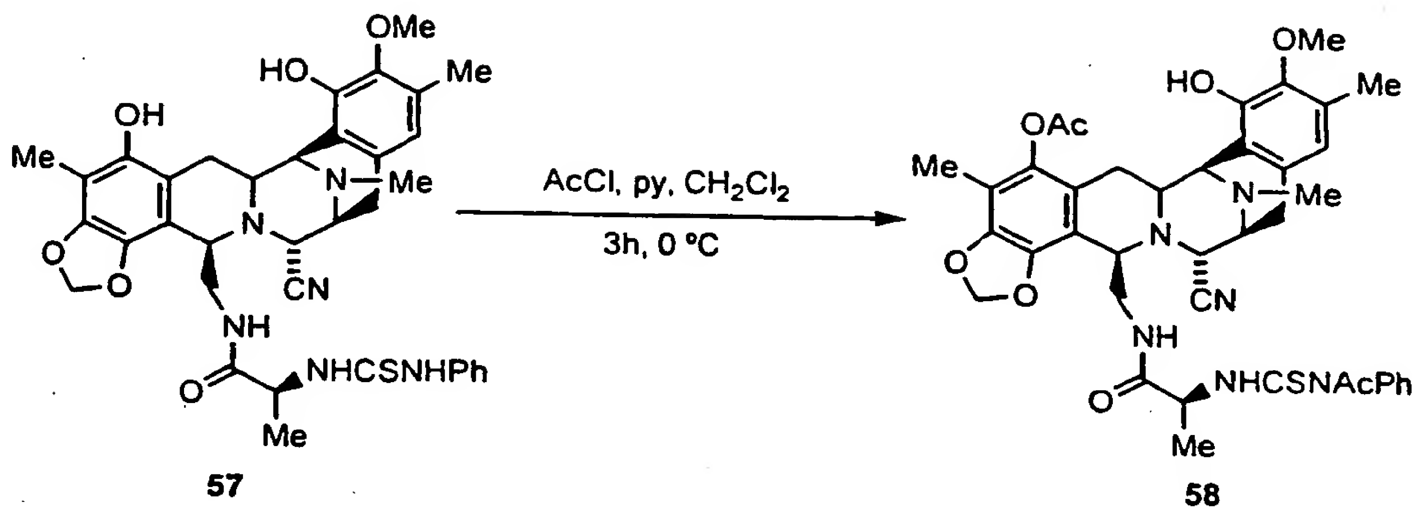
Example 51



To a solution of **56** (10 mg, 0.018 mmol) in CH_2Cl_2 (0.4 ml), phenyl isothiocyanate (13 ml, 0.109 mmol) was added and the reaction was stirred at 23° C for 1.5h. The mixture was concentrated *in vacuo* and the residue was purified by flash column chromatography (SiO_2, gradient Hexane to 1:1 hexane:ethyl acetate) to afford **57** (8 mg, 65%) as a white solid.

Rf: 0.57 (ethyl acetate:methanol10:1).
^{1}H NMR (300 MHz, $CDCl_3$): δ 7.88 (bs, 1H), 7.41-7.36 (m, 2 h), 7.27-7.22 (m, 1H), 7.02-7.00 (d, *J*= 7.8 Hz, 2 h), 6.71 (d, *J*= 7.2 Hz, 1H), 6.31 (s, 1H), 6.17 (bs, 1H), 5.93 (d, *J*=1.2 Hz, 1H), 5.83 (d, *J*= 1.2 Hz, 1H), 5.55 (bs, 1H), 5.20-5.17 (m, 1H), 4.16 (d, *J*= 1.8 Hz, 1H), 4.05 (bs, 1H), 4.02 (d, *J*= 2.4 Hz, 1H), 3.79 (s, 3H), 3.75-3.71 (m, 1H), 3.35 (d, *J*= 7.8 Hz, 1H), 3.28-3.19 (m, 2 h), 3.12-2.97 (m, 2 h), 2.50 (d, *J*=18.3 Hz, 1H), 2.32 (s, 3H), 2.21 (s, 3H), 2.15-2.09 (dd, J_1= 11.4 Hz, J_2= 15.9 Hz, 1H), 1.95 (s, 3H), 0.88 (d, *J*=6.9 Hz, 3H).
^{13}C NMR (75 MHz, $CDCl_3$): δ 178.5, 171.7, 147.2, 145.0, 144.3, 143.3, 137.0, 135.7, 130.6, 130.4, 129.6, 127.5, 124.3, 120.6, 117.7, 117.2, 115.3, 112.1, 108.3, 100.9, 60.9, 59.5, 56.7, 56.5, 56.2, 55.2, 54.1, 41.7, 41.1, 26.3, 25.4, 18.5, 15.8, 9.0.
ESI-MS m/z: Calcd. for $C_{36}H_{40}N_6O_6S$: 684.81. Found $(M+H)^+$: 685.3.

Example 52



To a solution of **57** (45 mg, 0.065 mmol) in CH_2Cl_2 (0.5 ml), acetyl chloride (4.67 ml, 0.065 mmol) and pyridine (5.3 ml, 0.065 mmol) were added at 0 °C. The reaction mixture was stirred for 3h and then, the solution was diluted with CH_2Cl_2 (10 ml) and washed with 0.1 N HCl (5 ml). The organic layer was dried over sodium sulphate, filtered, and the solvent was eliminated under reduced pressure. The residue was purified by flash column chromatography (RP-18, CH_3CN: H_2O 40:60) to afford **58** (14 mg, 28%) as a white solid.

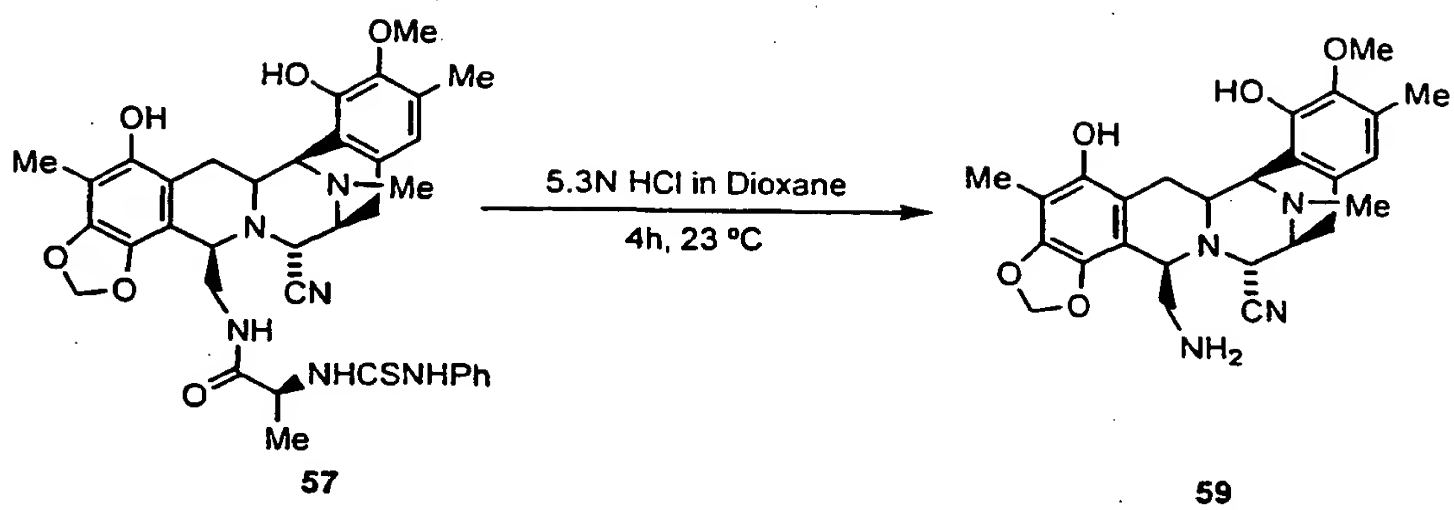
Rf: 0.34 (CH_3CN: H_2O 7:15).

^1H NMR (300 MHz, $CDCl_3$). δ 11.90 (d, *J*= 6.6 Hz, 1H), 7.45-7.40 (m, 3H), 7.18-7.15 (m, 2 h), 6.58 (s, 1H), 6.00 (d, *J*= 1.2 Hz, 1H), 5.89 (d, *J*= 1.2 Hz, 1H), 5.70 (s, 1H), 5.37 (t, *J*= 4.8 Hz, 1H), 4.48 (m, 1H), 4.23 (bs, 1H), 4.07 (bs, 2 h), 3.85-3.75 (m, 1H), 3.70 (s, 3H), 3.46-3.41 (m, 2 h), 3.24-3.20 (m, 1H), 3.00-2.95 (m, 1H), 2.87-2.75 (m, 1H), 2.31 (s, 3H), 2.28 (s, 3H), 2.24 (s, 3H), 2.00 (s, 3H), 1.85 (dd, J_1= 11.4 Hz, J_2= 15.6 Hz, 1H), 1.66 (s, 3H), 0.82 (d, *J*= 6.0 Hz, 3H).

^{13}C NMR (75 MHz, $CDCl_3$)): δ 182.6, 174.3, 171.0, 146.6, 144.6, 142.7, 142.3, 140.7, 140.2, 131.3, 129.8, 129.3, 128.9, 128.8, 121.5, 120.4, 117.3, 116.6, 112.8, 112.0, 111.3, 101.5, 60.5, 59.0, 57.6, 56.2, 55.9, 55.3, 55.1, 41.6, 39.4, 27.8, 26.5, 24.8, 20.2, 17.1, 15.5, 9.3.

ESI-MS m/z: Calcd. for $C_{40}H_{44}N_6O_8S$: 768.88. Found $(M+H)^+$: 769.2.

Example 53



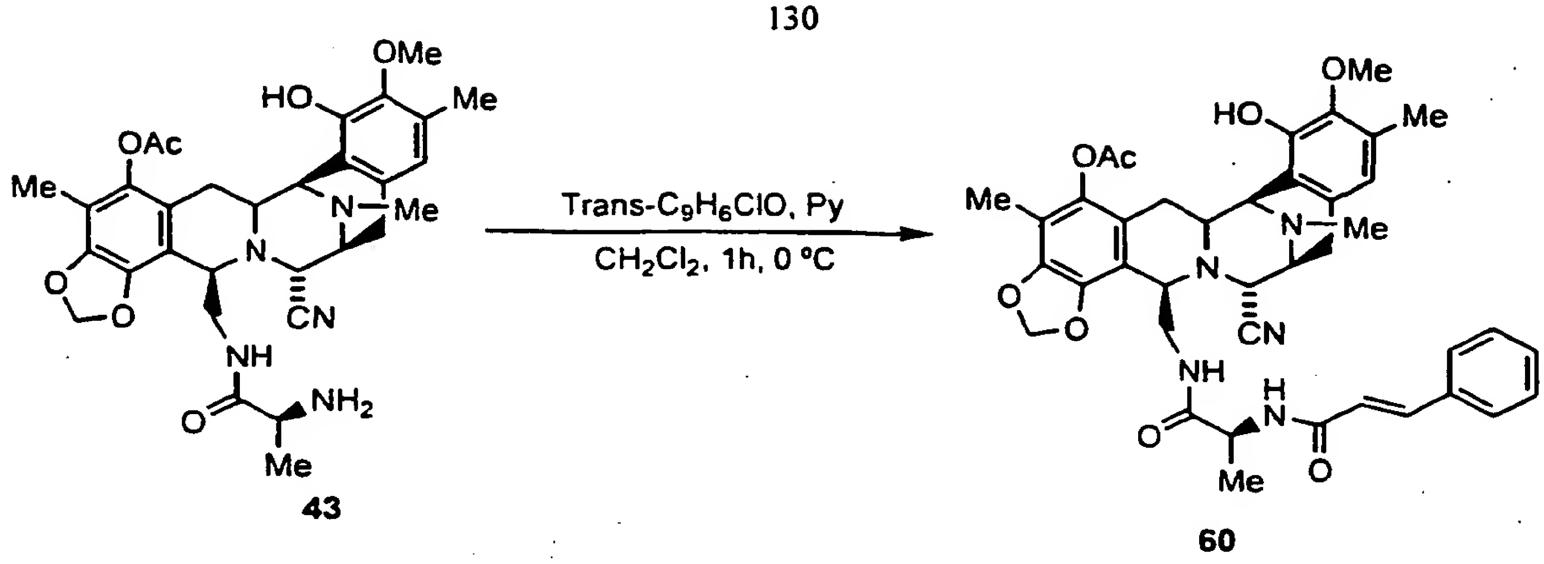
A solution of **57** (130 mg, 0.189 mmol) in dioxane (1 ml), 5.3N HCl/dioxane (1.87 ml) was added and the reaction was stirred at 23 °C for 4h. Then, CH_2Cl_2 (15 ml) and H_2O (10 ml) were added to this reaction and the organic layer was decanted. The aqueous phase was basified with saturated aq sodium bicarbonate (60 ml) (pH = 8) at 0 °C and then, extracted with ethyl acetate (2x50 ml). The combined organic extracts were dried (sodium sulphate), and concentrated *in vacuo* to afford **59** (63 mg, 70%) as a white solid.

Rf: 0.15 (ethyl acetate:methanol5:1).

^1H NMR (300 MHz, $CDCl_3$). δ 6.67 (s, 1H), 5.99 (d, J= 0.9 Hz, 1H), 5.91 (d, J= 1.2 Hz, 1H), 5.10 (bs, 1H), 4.32 (d, J= 7.2 Hz, 1H), 4.25 (dd, J_1= 3.6 Hz, J_2= 9.3 Hz, 1H), 3.7 (s, 3H), 3.71-3.64 (m, 2 h), 3.50 (dd, J_1= 2.4 Hz, J_2= 15.9 Hz, 1H), 3.42-3.37 (m, 2 h), 3.16 (dd, J_1=3.6 Hz, J_2= 12.9 Hz, 1H), 2.57 (dd, J_1= 9.3 Hz, J_2= 12.9 Hz, 1H), 2.27 (s, 3H), 2.11 (s, 3H), 1.91 (dd, J_1= 12.0 Hz, J_2= 15.9 Hz, 1H).

ESI-MS m/z: Calcd. for $C_{26}H_{30}N_4O_5$: 478.5. Found $(M+H)^+$: 479.3.

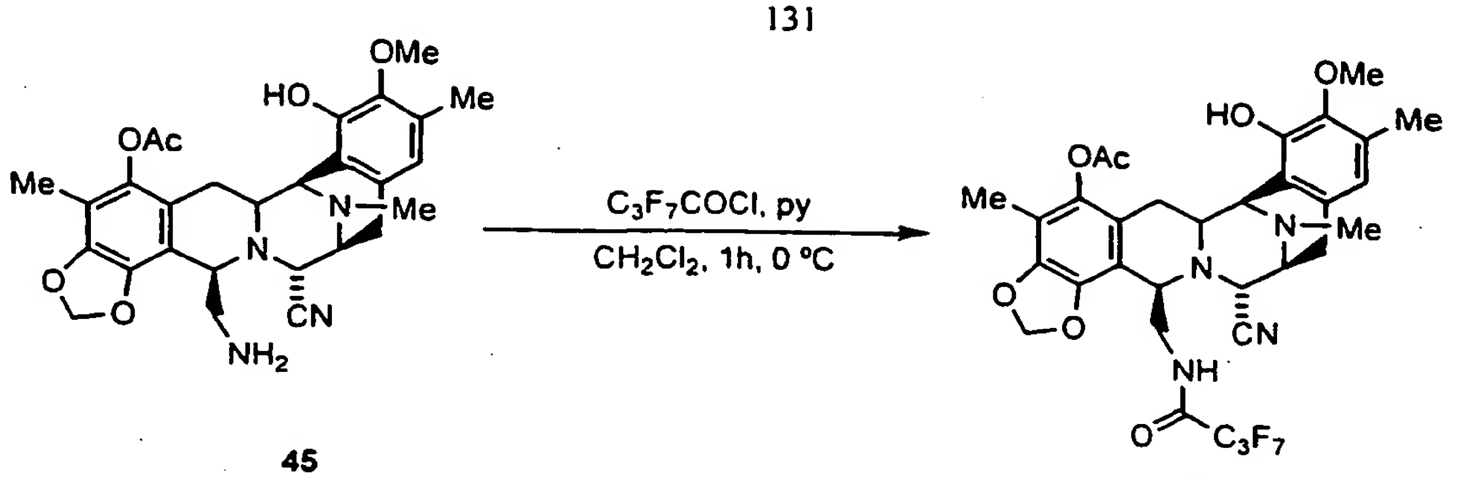
Example 54



A solution of **43** (20 mg, 0.0338 mmol) in CH_2Cl_2 (0.3 ml), cinnamoyl chloride (5.63 mg , 0.0338 mmol) and pyridine (2.73 ml, 0.0338 mmol) were added at 0 °C. The reaction mixture was stirred for 1h and then, the solution was diluted with CH_2Cl_2 (10 ml) and washed with 0.1 N HCl (5 ml). The organic layer was dried over sodium sulphate, filtered, and the solvent was eliminated under reduced pressure. The residue was purified by flash column chromatography (SiO_2, EtOAc:MeOH 20:1) to afford **60** (22 mg, 90%) as a white solid.

Rf: 0.56 (EtOAc:MeOH 5:1).
^1H NMR (300 MHz, $CDCl_3$). δ 7.51 (s, 1H), 7.50-7.47 (m, 2H), 7.36-7.35 (m, 2H), 6.43 (s, 1H), 6.36 (brd, J= 15.9 Hz, 2H), 6.01 (d, J= 1.5 Hz, 1H), 5.90 (brd, J= 1.5 Hz, 2H), 5.42 (t, J= 6.0 Hz 1H), 4.12-4.07 (m, 3H), 3.96-3.95 (m, 1H), 3.73 (bs, 3H), 3.58 (bs, 2H), 3.39 (d, J= 8.7 Hz, 1H), 3.25 (d, J= 11.7 Hz, 1H), 3.0 (dd, J_1= 7.5 Hz, J_2= 17.7 Hz, 1H), 2.78 (d, J= 15.9 Hz, 1H), 2.67 (d, J= 16.5 Hz, 1H), 2.29 (s, 6H), 2.23 (s, 3H), 1.99 (s, 3H), 1.82 (dd, J_1= 11.4 Hz, J_2= 15.6 Hz, 1H), 0.83 (d, J= 6.0 Hz, 3H).
^{13}C NMR (75 MHz, $CDCl_3$)): δ 172.0, 165.0, 146.9, 144.6, 143.1, 141.0, 140.5, 134.8, 131.0, 129.7, 129.1, 128.8, 127.8, 125.5, 123.8, 123.0, 121.1, 120.5, 117.7, 116.9, 112.8, 112.0, 101.9, 60.6, 59.2, 57.1, 56.4, 55.9, 55.3, 48.8, 41.7, 40.0, 26.5, 25.1, 20.3, 18.5, 15.7, 9.3.
ESI-MS m/z: Calcd. for $C_{40}H_{43}N_5O_8$: 721.8. Found $(M+H)^+$: 722.3.

Example 55



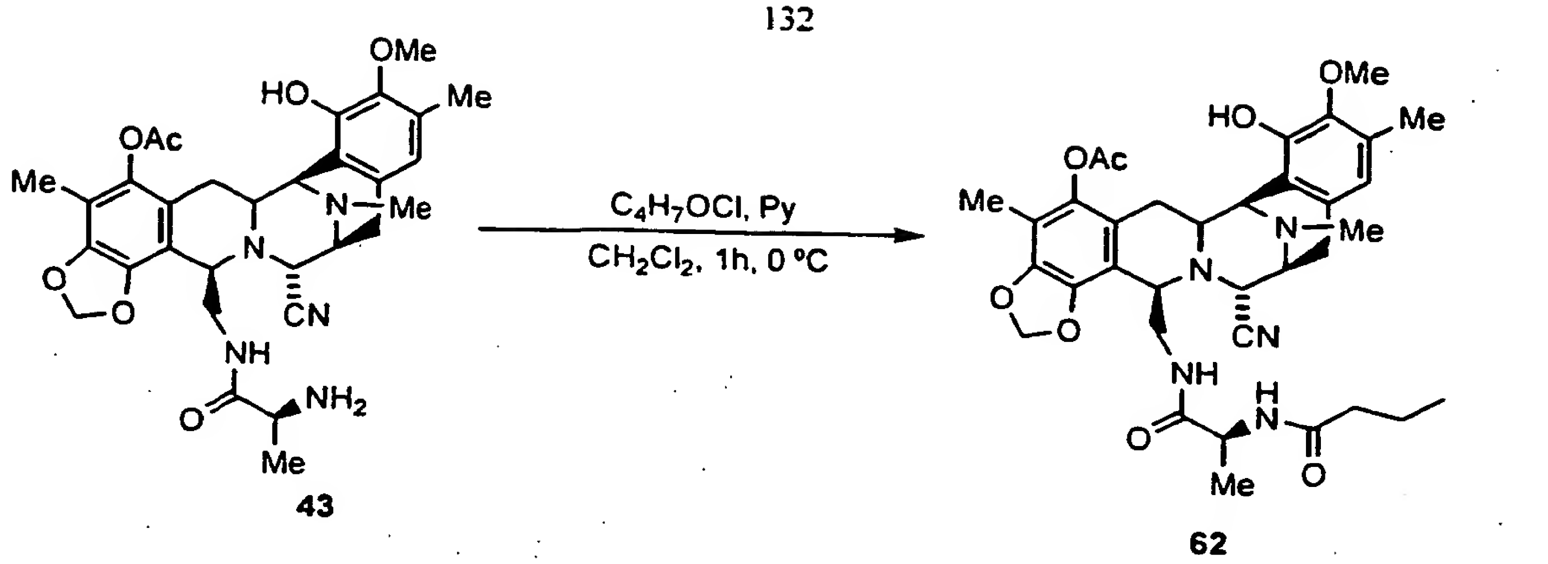
A solution of **45** (19 mg, 0.0364 mmol) in CH_2Cl_2 (0.3 ml), heptafluorobutyryl chloride (5.44 ml, 0.0364 mmol) and pyridine (2.95 ml, 0.0364 mmol) were added at 0 °C. The reaction mixture was stirred for 1h and then, the solution was diluted with CH_2Cl_2 (10 ml) and washed with 0.1 N HCl (5 ml). The organic layer was dried over sodium sulphate, filtered, and the solvent was eliminated under reduced pressure. The residue was purified by flash column chromatography (SiO_2, EtOAc:MeOH 20:1) to afford **61** (11.7 mg, 45%) as a white solid.

Rf: 0.76 (EtOAc:MeOH 5:1).

^1H NMR (300 MHz, $CDCl_3$) δ 6.46 (s, 1H), 6.12 (bs, 1H), 5.98 (d, J= 1.2 Hz, 1H), 5.93 (d, J= 1.2 Hz, 1H), 5.72 (bs, 1H), 4.13-4.11 (m, 2H), 4.0 (d, J= 2.4 Hz, 1H), 3.98-3.96 (m, 1H), 3.73 (s, 3H), 3.39 (d, J= 7.5 Hz, 1H), 3.39-3.28 (m, 2H), 3.09 (dd, J_1= 8.1 Hz, J_2= 18.0 Hz, 1H), 2.80 (d, J= 16.2 Hz, 1H), 2.46 (d, J= 18.3 Hz, 1H), 2.32 (s, 6H), 2.21 (s, 3H), 1.99 (s, 3H), 1.80 (dd, J_1= 12.0 Hz, J_2= 16.2 Hz, 1H).

ESI-MS m/z: Calcd. for $C_{32}H_{31}F_7N_4O_7$: 716.6. Found $(M+H)^+$: 717.2.

Example 56



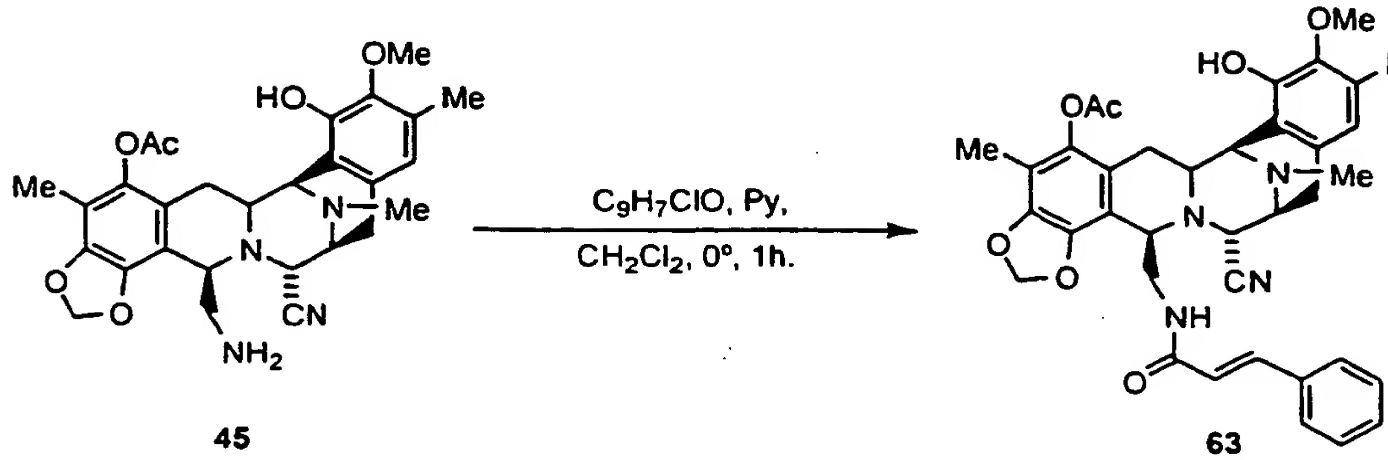
A solution of **43** (24 mg, 0.04 mmol) in CH_2Cl_2 (0.3 ml), butyryl chloride (4.15 ml, 0.04 mmol) and pyridine (3.28 ml, 0.04 mmol) were added at 0 °C. The reaction mixture was stirred for 1h and then, the solution was diluted with CH_2Cl_2 (10 ml) and washed with 0.1 N HCl (5 ml). The organic layer was dried over sodium sulphate, filtered, and the solvent was eliminated under reduced pressure. The residue was purified by flash column chromatography (SiO_2, EtOAc:MeOH 20:1) to afford **62** (24 mg, 90%) as a white solid.

Rf: 0.35 (EtOAc:MeOH 5:1).

^1H NMR (300 MHz, $CDCl_3$) δ 6.47 (s, 1H), 6.10 (d, J= 6.5 Hz, 1H), 6.0 (d, J= 1.5 Hz, 1H), 5.91 (d, J= 1.5 Hz, 1H), 5.86 (bs, 1H), 5.31 (d, J= 6.9 Hz, 1H), 4.11-4.06 (m, 3H), 3.85-3.81 (m, 1H), 3.75 (s, 3H), 3.59-3.53 (m, 2H), 3.38 (d, J= 7.5 Hz, 1H), 3.27-3.22 (m, 1H), 3.0 (dd, J_1= 7.8 Hz, J_2= 17.4 Hz, 1H), 2.79 (d, J= 15.3 Hz, 1H), 2.63 (d, J= 17.7 Hz, 1H), 2.31 (s, 3H), 2.0 (s, 3H), 1.80 (dd, J_1= 12.0 Hz, J_2= 15.9 Hz, 1H), 1.58 (q, J= 7.2 Hz, 2H), 0.89 (t, J= 7.2 Hz, 3H), 0.76 (d, J= 6.6 Hz, 3H).

ESI-MS m/z: Calcd. for $C_{35}H_{43}N_5O_8$: 661.64. Found $(M+H)^+$: 662.3

Example 57



45 63

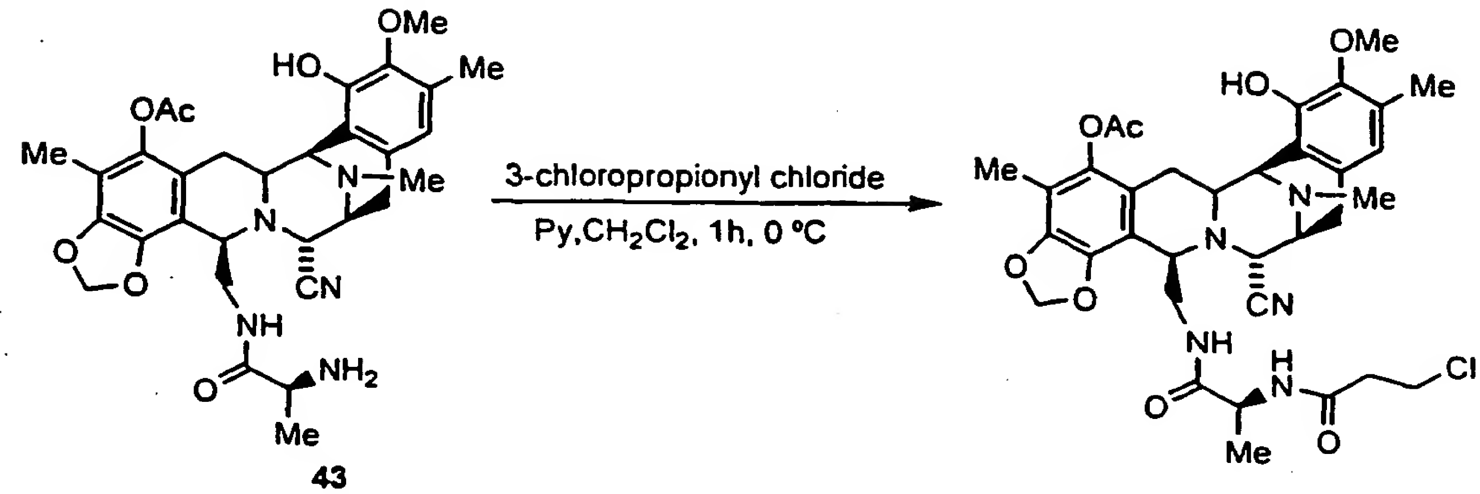
A solution of **43** (19 mg, 0.0364 mmol) in CH_2Cl_2 (0.3 ml), cinnamoyl chloride (6.06 mg , 0.0364 mmol) and pyridine (2.95 ml, 0.0364 mmol) were added at 0 °C. The reaction mixture was stirred for 1h and then, the solution was diluted with CH_2Cl_2 (10 ml) and washed with 0.1 N HCl (5 ml). The organic layer was dried over sodium sulphate, filtered, and the solvent was eliminated under reduced pressure. The residue was purified by flash column chromatography (SiO_2, EtOAc:MeOH 20:1) to afford **63** (20.1 mg, 85%) as a white solid.

Rf: 0.65 (EtOAc:MeOH 5:1).

^1H NMR (300 MHz, $CDCl_3$) δ 7.39-7.29 (m, 5H), 6.42, (s, 1H), 6.01 (d, J= 1.5 Hz, 1H), 5.92 (d, J= 1.5 Hz, 1H), 5.73 (bs, 1H), 5.24 (t, J= 6.8 Hz, 1H), 4.12-4.08 (m, 3H), 3.66-3.64 (m, 2H), 3.58 (bs, 3H), 3.36 (d, J= 8.7 Hz, 1H), 3.29 (d, J= 12.0 Hz, 1H), 2.98 (dd, J_1= 8.1 Hz, J_2= 18 Hz, 1H), 2.33 (s, 6H), 2.29 (s, 3H), 2.01 (s, 3H), 1.84 (dd, J_1= 12.0 Hz, J_2= 15.9 Hz, 1H).

ESI-MS m/z: Calcd. for $C_{37}H_{38}N_4O_7$: 650.72. Found $(M+H)^+$: 651.2.

Example 58



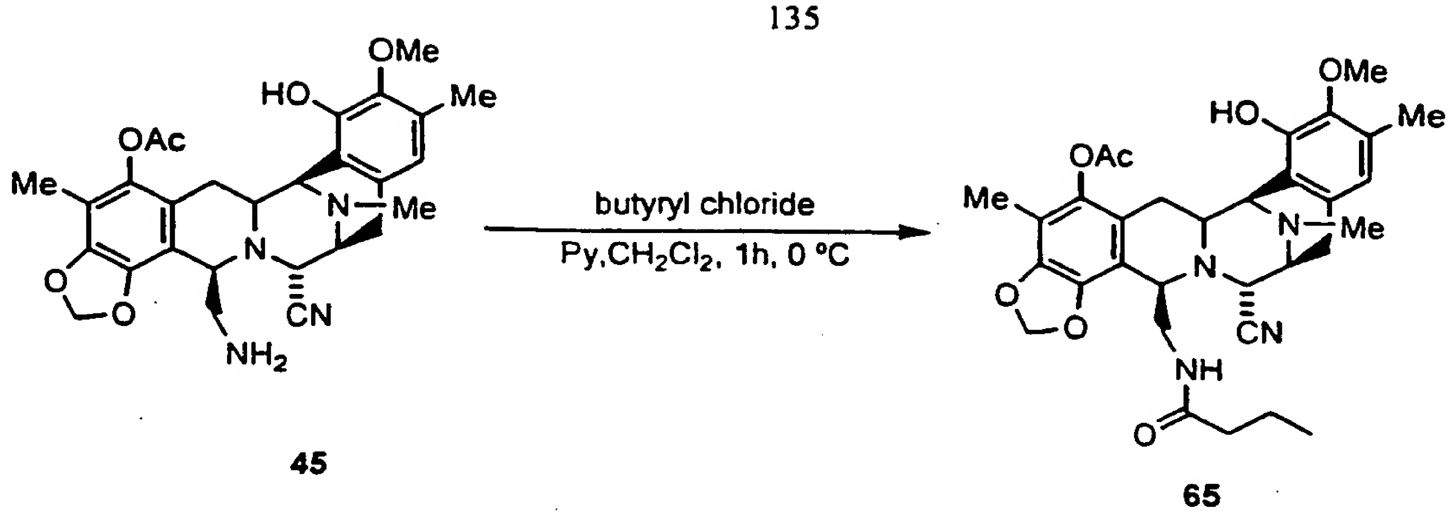
43 64

A solution of **43** (20 mg, 0.0338 mmol) in CH_2Cl_2 (0.3 ml), 3-chloropropionyl chloride (3.22 ml , 0.0338 mmol) and pyridine (2.73 ml, 0.0338 mmol) were added at 0 °C. The reaction mixture was stirred for 1h and then, the solution was diluted with CH_2Cl_2 (10 ml) and washed with 0.1 N HCl (5 ml). The organic layer was dried over sodium sulphate, filtered, and the solvent was eliminated under reduced pressure. The residue was purified by flash column chromatography (SiO_2, EtOAc:MeOH 20:1) to afford **64** (20.5 mg, 89%) as a white solid.

Rf: 0.32 (EtOAc:Hexane 5:1).

^{1}H NMR (300 MHz, $CDCl_3$) δ 6.48 (s, 3H), 6.28 (m, 1H), 5.99 (d, J= 1.2 Hz, 1H), 5.91 (d, J= 1.2 Hz, 1H), 5.86 (bs, 1H), 5.31 (m, 1H), 4.08-4.07 (m, 3H), 3.75 (s, 3H), 3.72-3.53 (m, 5H), 3.39 (d, J= 8.1 Hz, 1H), 3.24 (d, J= 12.0 Hz, 1H), 3.00 (dd, J_1= 8.1 Hz, J_2= 18.0 Hz, 1H), 2.79 (d, J= 13.5 Hz, 1H), 2.50 (t, J= 6.3 Hz, 2H), 2.32 (s, 3H), 2.28 (s, 3H), 2.25 (s, 3H), 2.0 (s, 3H), 1.79 (dd, J_1= 12.3 Hz, J_2= 14.8 Hz, 1H), 0.81 (d, J= 6.3 Hz, 3H).

Example 59



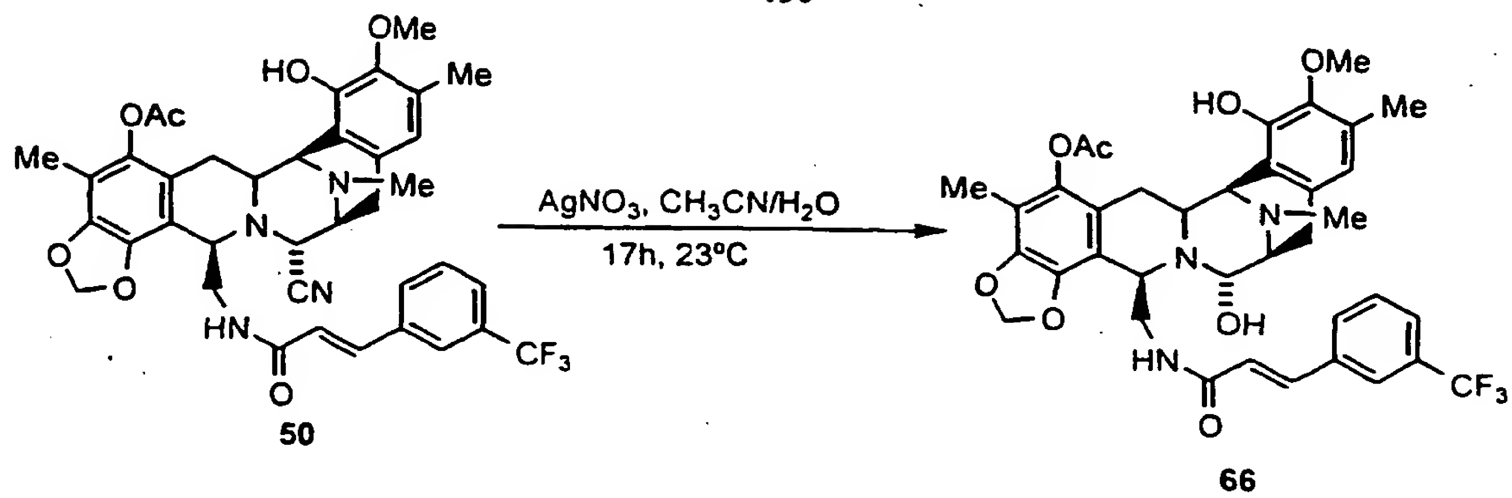
A solution of **43** (19 mg, 0.0364 mmol) in CH_2Cl_2 (0.3 ml), butyryl chloride (3.78 ml , 0.0364 mmol) and pyridine (2.95 ml, 0.0364 mmol) were added at 0 °C. The reaction mixture was stirred for 1h and then, the solution was diluted with CH_2Cl_2 (10 ml) and washed with 0.1 N HCl (5 ml). The organic layer was dried over sodium sulphate, filtered, and the solvent was eliminated under reduced pressure. The residue was purified by flash column chromatography (SiO_2, EtOAc:MeOH 20:1) to afford **64** (19 mg, 87%) as a white solid.

Rf: 0.60 (EtOAc:MeOH 5:1).

^{1}H NMR (300 MHz, $CDCl_3$) δ 6.50 (s, 1H), 5.98 (d, *J*= 1.5 Hz, 1H), 5.91 (d, *J*= 1.5 Hz, 1H), 5.75 (s,1H), 5.01 (t, *J*= 6.4 Hz, 1H), 4.10 –4.09 (m, 1H), 4.06 (d, *J*= 2.1 Hz, 1H), 4.03-4.02 (m, 1H), 3.76 (s, 3H), 3.67-3.60 (m, 1H), 3.42-3.35 (m, 2H), 3.29 (d, *J*= 12.0 Hz, 1H), 3.02 (dd, J_1= 7.8 Hz, J_2= 17.7 Hz, 1H), 2.79 (d, *J*= 14.1 Hz, 1H), 2.56 (d, *J*= 18.3 Hz, 1H), 2.32 (s, 3H), 2.31 (s, 3H), 2.25 (s, 3H), 1.78 (dd, J_1= 12.0 Hz, J_2= 15.9 Hz, 1H), 1.63 (s, 3H), 1.53-1.46 (m, 2H), 1.28-1.16 (m, 2H), 0.68 (t, *J*= 7.2 Hz, 3H).

ESI-MS m/z: Calcd. for $C_{32}H_{38}N_4O_7$: 590.67. Found $(M+H)^+$: 591.2.

Example 60



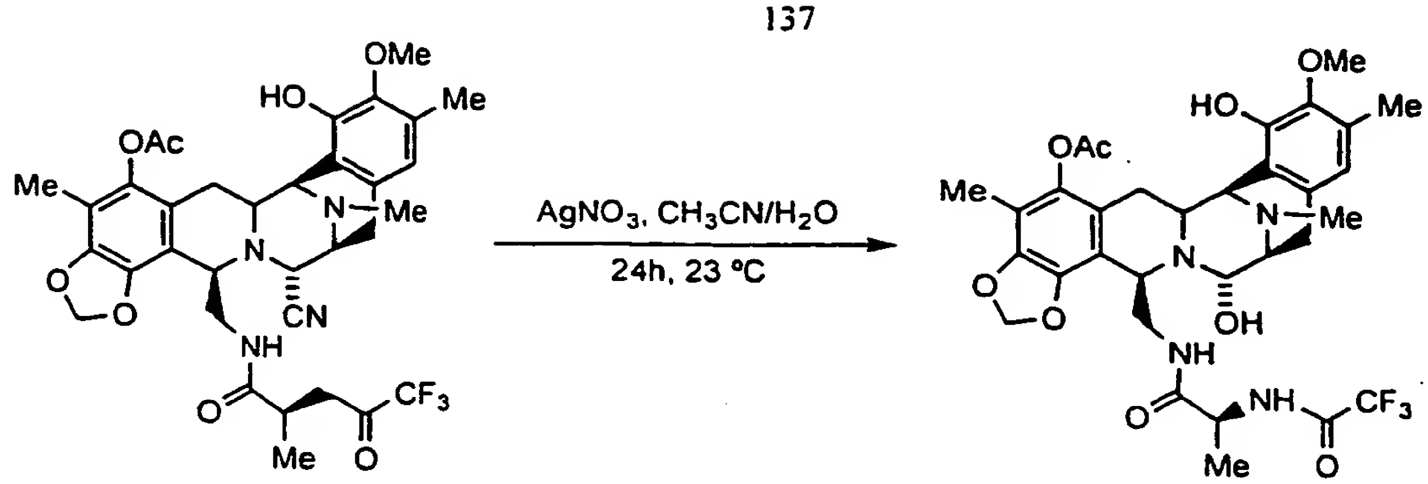
To a solution of **50** (31.7 mg, 0.044 mmol) in CH_3CN/H_2O (1.5 ml/0.5 ml), $AgNO_3$ (225 mg, 1.32 mmol) was added and the reaction was stirred at 23°C for 17 h. Then brine (10 ml) and Aq sat $NaHCO_3$ (10 ml) were added at 0°C and the mixture was stirred for 15 min, filtered through a pad of celite and washed with CH_2Cl_2 (20 ml). The solution was decanted and the organic layer was dried and concentrated *in vacuo*. The residue was purified by flash column chromatography (SiO_2, EtOAc:MeOH 5:1) to afford **66** (16 mg, 51%) as a white solid.

Rf: 0.26 (EtOAc:MeOH 5:1).

^1H NMR (300 MHz, $CDCl_3$) δ 7.66-7.42 (m, 4H), 7.20 (bs, 1H), 6.44 (s, 1H), 5.97 (b, *J*= 1.2 Hz, 1H), 5.90 (d, *J*= 1.2 Hz, 1H), 5.76 (bs, 1H), 5.28 (bs, 1H), 4.54 (bs, 1H), 4.43 (bs, 1H), 4.00 (bs, 1H), 3.68-3.57 (m, 4H), 3.47 (d, *J*= 3.3 Hz, 1H), 3.40 (d, *J*= 11.7 Hz, 1H), 3.17 (d, *J*= 6.9 Hz, 1H), 2.92 (dd, J_1= 8.1 Hz, J_2= 17.7 Hz, 1H), 2.74 (d, *J*= 17.1 Hz, 1H), 2.48 (d, *J*= 18.6 Hz, 1H), 2.32 (s, 6H), 2.28 (s, 3H), 1.99 (s, 3H), 1.76 (dd, J_1= 12.0 Hz, J_2= 16.2 Hz, 1H).

ESI-MS *m/z*: Calcd. for $C_{37}H_{38}F_3N_3O_8$: 709. Found (M^+-17): 692.3.

Example 61



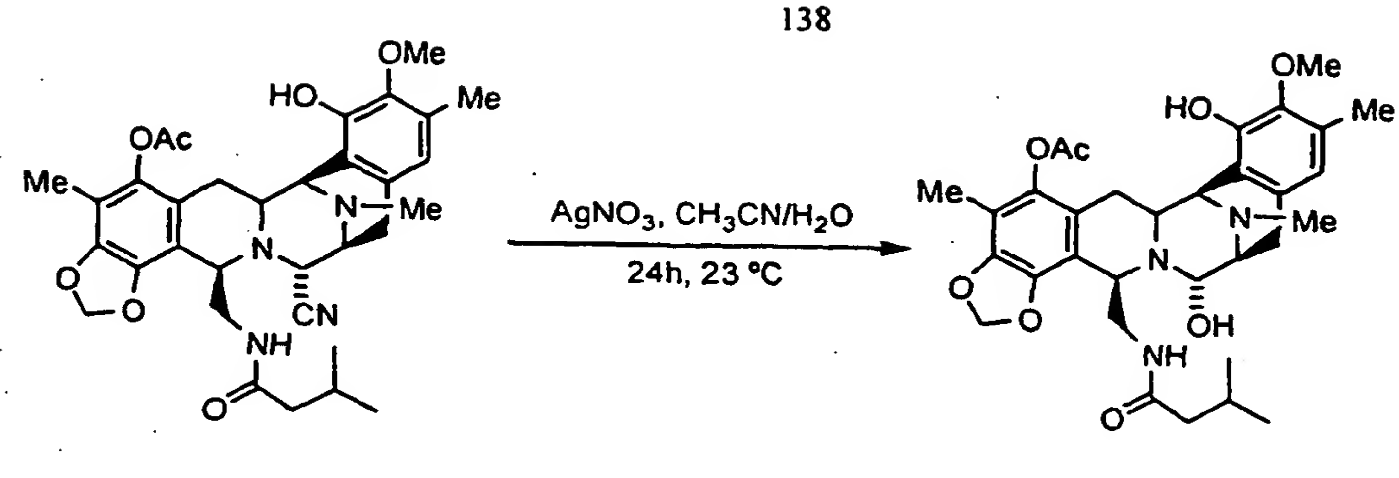
To a solution of **53** (57 mg, 0.0828 mmol) in CH_3CN/H_2O (1.5 mL/0.5 ml), $AgNO_3$ (650 mg, 3.81 mmol) was added and the reaction was stirred at 23°C for 24 h. Then, brine (10 ml) and Aq sat $NaHCO_3$ (10 ml) were added at 0°C and the mixture was stirred for 15 min, filtered through a pad of celite and washed with CH_2Cl_2 (20 ml). The solution was decanted and the organic layer was dried and concentrated *in vacuo*. The residue was purified by flash column chromatography (SiO_2, EtOAc:MeOH 5:1) to afford **67** (28 mg, 50%) as a white solid.

Rf: 0.28 (EtOAc:MeOH 10:1).

^1H NMR (300 MHz, $CDCl_3$) δ 6.47 (s, 1H), 5.97 (s, 1H), 5.88 (s, 1H), 5.35 (bs, 1H), 4.51 (bs, 1H), 4.41 (bs, 1H), 4.12-4.05 (m, 1H), 4.00 (d, J= 2.7 Hz, 1H), 3.77 (s, 3H), 3.64 (bs, 1H), 3.46 (d, J= 3.3 Hz, 1H), 3.34 (d, J= 11.4 Hz, 1H), 3.18 (d, J= 7.5 Hz, 1H), 2.95 (dd, J_1= 8.4 Hz, J_2= 18.3 Hz, 1H), 2.70 (d, J= 15.6 Hz, 1H), 2.48 (d, J= 17.7 Hz, 1H), 2.28 (s, 3H), 2.27 (s, 3H), 2.26 (s, 3H), 1.98 (s, 3H), 1.68 (dd, J_1= 12 Hz, J_2= 15.6 Hz, 1H), 0.86 (d, J= 6.3 Hz, 3H).

ESI-MS m/z: Calcd. for $C_{32}H_{37}F_3N_4O_9$: 678.66. Found (M^+-17): 661.2.

Example 62



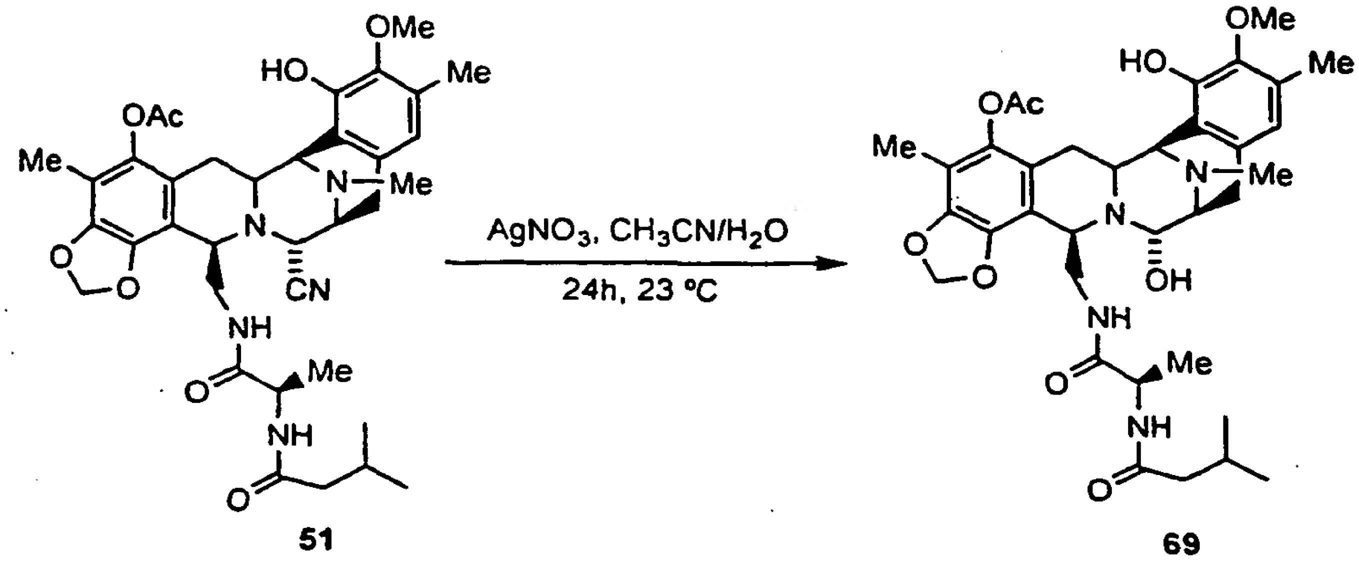
48 **68**

To a solution of **48** (32 mg, 0.0529 mmol) in CH_3CN/H_2O (1.5 ml/0.5 ml), $AgNO_3$ (270 mg, 1.58 mmol) was added and the reaction was stirred at 23°C for 24 h. Then, brine (10 ml) and Aq sat $NaHCO_3$ (10 ml) were added at 0°C and the mixture was stirred for 15 min, filtered through a pad of celite and washed with CH_2Cl_2 (20 ml). The solution was decanted and the organic layer was dried and concentrated *in vacuo*. The residue was purified by flash column chromatography (SiO_2, EtOAc:MeOH 5:1) to afford **68** (18 mg, 56%) as a white solid.

Rf: 0.40 (EtOAc:MeOH 5:1).

^1H NMR (300 MHz, $CDCl_3$) δ 6.50 (s, 1H), 5.95 (d, *J*= 1.2 Hz, 1H), 5.88 (d, *J*= 1.2 Hz, 1H), 5.23 (d, *J*= 6.9 Hz, 1H), 4.45 (d, *J*= 3.3 Hz, 1H), 4.38 (s, 1H), 4.01 (d, *J*= 2.4 Hz, 1H), 3.78 (m, 1H), 3.77 (s, 3H), 3.41-3.37 (m, 1H), 3.17-3.15 (m, 1H), 2.96 (dd, J_1= 7.8 Hz, J_2= 18.0 Hz, 1H), 2.70 (d, *J*= 15.3 Hz, 1H), 2.40 (d, *J*= 18.0 Hz, 1H), 2.30 (s, 6H), 2.27 (s, 3H), 1.76-1.65 (m, 1H), 1.35-1.25 (m, 2H), 0.89-0.82 (m, 1H), 0.69 (d, *J*= 6.6 Hz, 3H), 0.58 (d, *J*= 6.6 Hz, 3H)

Example 63

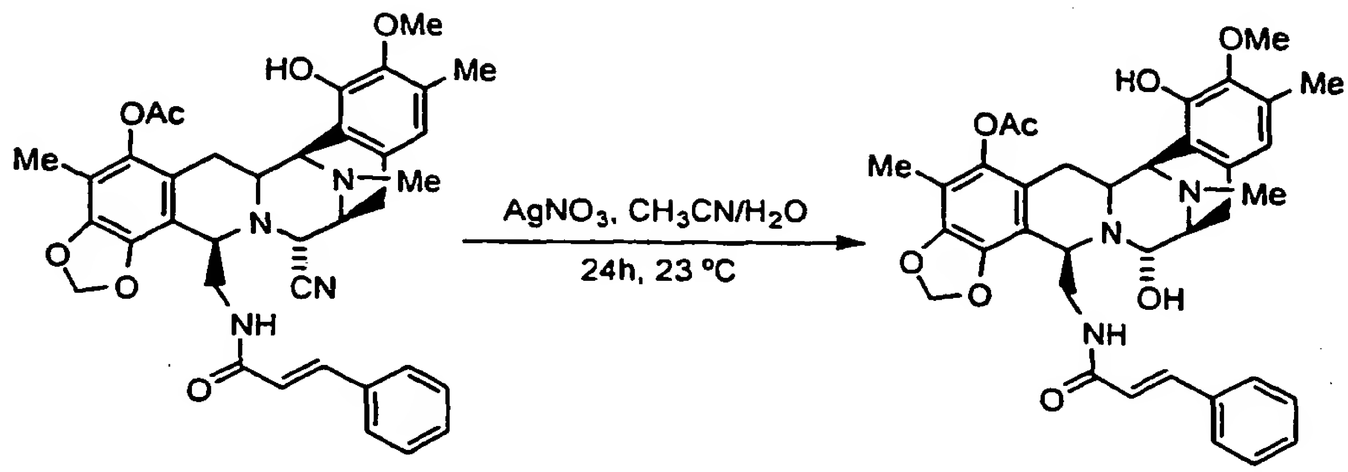


To a solution of **51** (27 mg, 0.04 mmol) in CH_3CN/H_2O (1.5 ml/0.5 ml), $AgNO_3$ (204 mg, 1.19 mmol) was added and the reaction was stirred at 23°C for 24 h. Then, brine (10 ml) and Aq sat $NaHCO_3$ (10 ml) were added at 0°C and the mixture was stirred for 15 min, filtered through a pad of celite and washed with CH_2Cl_2 (20 ml). The solution was decanted and the organic layer was dried and concentrated *in vacuo*. The residue was purified by flash column chromatography (SiO_2, EtOAc:MeOH 5:1) to afford **69** (10 mg, 38%) as a white solid.

Rf: 0.38 (EtOAc:MeOH 5:1).

^{1}H NMR (300 MHz, $CDCl_3$) δ 6.48 s, 1H), 6.16 (bs, 1H), 5.98 (d, *J*= 1.5 Hz, 1H), 5.89 (d, *J*= 1.5 Hz, 1H), 5.33 (t, *J*= 6.0 Hz, 1H), 4.50 (m, 1H), 4.40 (m, 1H), 4.11-4.09 (m, 1H), 4.00 (d, *J*= 2.6Hz, 1H), 3.78 (s, 3H), 3.41-3.32 (m, 3H), 3.18 (d, *J*= 8.4 Hz, 1H), 2.94 (dd, J_1= 8.4 Hz, J_2= 18.3 Hz, 1H), 2.70 (d, *J*= 14.4 Hz, 1H), 4.45 (d, *J*= 18.3 Hz, 1H), 2.31 (s, 3H), 2.28 (s, 3H), 2.27 (s, 3H), 2.04 (s, 3H), 2.00-1.86 (m, 3H), 1.73 (m, 1H), 0.87 (d, *J*= 6.3 Hz, 6H).

Example 64



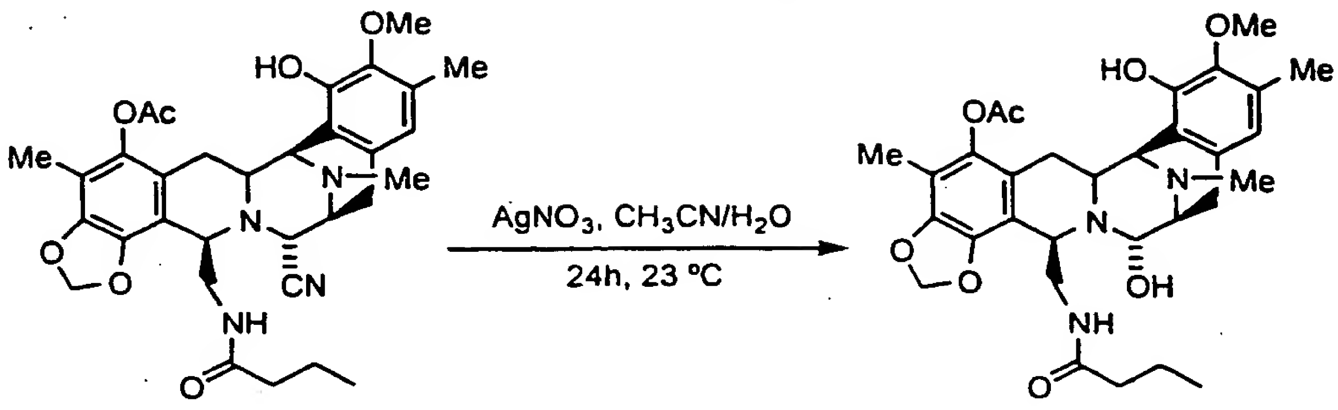
63 70

To a solution of **63** (15 mg, 0.023 mmol) in CH_3CN/H_2O (1.5 ml/0.5 ml), $AgNO_3$ (118 mg, 0.691 mmol) was added and the reaction was stirred at 23°C for 24 h. Then, brine (10 ml) and Aq sat $NaHCO_3$ (10 ml) were added at 0°C and the mixture was stirred for 15 min, filtered through a pad of celite and washed with CH_2Cl_2 (20 ml). The solution was decanted and the organic layer was dried and concentrated *in vacuo*. The residue was purified by flash column chromatography (SiO_2, EtOAc:MeOH 5:1) to afford **70** (20.1 mg, 85%) as a white solid.

Rf: 0.43 (EtOAc:MeOH 5:1).

^{1}H NMR (300 MHz, $CDCl_3$) δ 7.38-7.28 (m, 5H), 6.48 (s, 1H), 5.98 (d, J=1.5 Hz, 1H), 5.91 (d, J=1.5 Hz, 1H), 5.75 (bs, 1H), 5.38 (brd, 1H), 5.30 (bs, 1H), 4.53 (m, 1H), 4.42 (m, 1H), 4.02 (d, J=2.7 Hz, 1H), 3.78-3.65 (m, 5H), 3.46-3.40 (m, 2H), 3.17 (d, J=7.8 Hz, 1H), 2.94 (dd, J_1=7.8 Hz, J_2=17.7 Hz, 1H), 2.73 (d, J=16.8 Hz, 1H), 2.45 (d, J=18.0 Hz, 1H), 2.31 (s, 6H), 2.28 (s, 3H), 1.97 (s, 3H), 1.77 (dd, J_1=12.0 Hz, J_2=15.3 Hz, 1H).

Example 65



65 71

To a solution of **65** (25 mg, 0.042 mmol) in CH_3CN/H_2O (1.5 ml/0.5 ml), $AgNO_3$ (215.56 mg, 1.269 mmol) was added and the reaction was stirred at 23°C for 24 h. Then, brine (10 ml) and Aq sat $NaHCO_3$ (10 ml) were added at 0°C and the mixture was stirred for 15 min, filtered through a pad of celite and washed with CH_2Cl_2 (20 ml). The solution was decanted and the organic layer was dried and concentrated *in vacuo*. The residue was purified by flash column chromatography (SiO_2, EtOAc:MeOH 5:2) to afford **71** (16mg, 65%) as a white solid.

Rf: 0.0.5 (EtOAc:MeOH 5:2).

^1H NMR (300 MHz, $CDCl_3$) δ 6.50 (s, 1H), 5.95 (d, *J*=1.5 Hz, 1H), 5.78 (s, 1H), 5.19 (bs, 1H), 4.45 (d, *J*=3.3 Hz, 1H), 4.37 (bs, 1H), 4.11 (brd, *J*=4.8 Hz, 1H), 4.01 (d, *J*=2.1 Hz, 1H), 3.76 (s, 1H), 3.71-3.69 (m, 1H), 3.49-3.35 (m, 1H), 3.24 (d, *J*=13.5 Hz, 1H), 3.15 (d, *J*=9.3 Hz, 1H), 2.95 (dd, J_1=8.1 Hz, J_2=17.7 Hz, 1H), 2.70 (d, *J*=15.6 Hz, 1H), 2.40 (d, *J*=18.0 Hz, 1H), 2.31 (s, 3H), 2.29 (s, 3H), 2.26 (s, 3H), 1.96 (s, 3H), 1.75-1.66 (m, 1H), 1.52-1.17 (m, 2H), 0.66 (t, *J*=7.2 Hz, 3H).

Fermentation Procedures

Example A

Seed medium YMP3 containing 1% glucose; 0.25% beef extract; 0.5% bacto-peptone; 0.25% NaCl; 0.8% $CaCO_3$ was inoculated with

0.1% of a frozen vegetative stock of the microorganism, strain A2-2 of *Pseudomonas fluorescens,* and incubated on a rotary shaker (250 rpm) at 27°C. After 30 h of incubation, the seed culture was added to a agitated-vessel fermentor with a production medium composed of 2% dextrose; 4% mannitol, 2% dried brewer's yeast (*Vitalevor® Biolux, Belgium*); 1% $(NH_4)_2SO_4$; 0.04% K_2HPO_4; 0.8 KCl; 0.001% $FeCl_3$; 0.1% L-Tyr; 0.8% CO_3Ca; 0.05% PPG-2000; 0.2% anti-foam silicone (ASSAF-100, RHODIA UK). The sterilisation was carried out at 122°C 30 minutes. The volume inoculated was a 2% (v/v). The temperature was 27°C (0 to 16h) and 24°C from 16h to final process (41 hours). The dissolve oxygen-pressure was upper to 25%. The pH was controlled at 6.0 with diluted sulphuric acid since 28 hours till final process. The overpressure was 0.5 bar. A 1% mannitol or sorbitol was added from 16 h to final process (for two days running) and 2% for three days fermentation-process.

After 41 or 64 hours, the fermentation broth must be extracted for recovery safracin B or KCN treatment in the clarified broth for recovery safracin B - cyano.

Example B

Obtention of safracin B cyano from the crude extract.

A clarification or filtration from the fermentation broth at pH 6 removes the solids. The clarified broth was adjusted a pH 9.5 with diluted sodium hydroxide and extracted twice with 2:1 (v/v) ethyl acetate, methylene chloride or butyl acetate. The extraction was carried out into an agitated-vessel during 20', the temperature of the mixture was maintained at 8 to 10°C. The two phases were separated by a liquid-liquid centrifuge. The organic phase was dried with sodium sulphate anhydrous or frozen and then filtered for removing ice. This

organic phase (ethyl acetate layer) was evaporated until obtention of an oil-crude extract.

Example C

Obtention of safracin B cyano from the clarified broth.

A clarification or filtration from the fermentation broth at pH 6 removes the solids. The clarified broth was adjusted at pH 3.9 with concentrated acetic acid. 0.5 grams per litre of KCN are added to the clarified broth an incubated at 20°C during 1 hour with agitation. Then, the temperature was decreased at 15°C and the pH was adjusted at 9.5 with diluted sodium hydroxide and extracted with 2:1.5 (v/v) ethyl acetate. The extraction was carried out into an agitated-vessel during 20 minutes, the temperature of the mixture was maintained at 8 to 10°C. The two phases were separated by a liquid-liquid centrifuge. The organic phase was dried with sodium sulphate anhydrous. This organic phase (ethyl acetate layer) was evaporated until obtention of an oil-crude extract. This extract was purified by flash column chromatography (SiO_2, gradient 20:1 to 10: to 5:1 ethyl acetate:methanol) to afford quantitatively compound 2 as a light yellow solid.

Rf: 0.55 (ethyl acetate:methanol5:1); .t_R= 19.9 min [HPLC, Delta Pack C4, 5μm, 300 A, 150x3 mm, λ=215 nm, flow= 0.7 ml/min, temp= 50°C, grad.: CH_3CN-aq. NaOAc (10mM) 85% - 70% (20')];
^{1}H NMR (300 Mhz, $CDCl_3$): δ 6.54 (dd, J_1 = 4.4Hz, J_2 = 8.4 Hz, 1H),6.44 (s, 1H), 4.12 (d, J = 2.4 Hz, 1H), 4.04 (d, J = 2.4 Hz, 1H), 4.00 (s, 3H), 3.87 (bs, 1H), 3.65 (ddd, J_1 = 1.5 Hz, J_2 = 8.7 Hz, J_3 = 9.9 Hz, 1H), 3.35 (br. D, J = 8.4 Hz, 1H), 3.15-2.96 (m, 4H), 2.92 (q, J = 7.2 Hz, 1H), 2.47 (d, J = 18.3 Hz, 1H), 2.29 (s, 3H), 2.18 (s, 3H) 1.83 (s, 3H), 1.64 (ddd, J_1 = 2.7 Hz, J_2 = 11.1 Hz, J_3 = 14.1 Hz, 1H), 0.79 (d, J = 7.2 Hz, 3H);

^{13}C NMR (75 Mhz, $CDCl_3$): δ 186.0 (q), 175.9 (q), 156.2 (q), 146.8 (q), 142.8 (q), 140.7 (q), 136.6 (q), 130.5 (q), 128.8 (q), 127.0 (q), 120.5 (s), 117.4 (q), 116.5 (q), 60.8 (t), 60.4 (s), 58.7 (t), 56.2 (s), 55.7 (s), 54.8 (s), 54.8 (s), 54.4 (s), 50.0 (s), 41.6 (t), 39.8 (d), 25.2 (d), 24.4 (d), 21.2 (t), 15.5 (t), 8.4 (t).

ESI-MS m/z: Calcd for $C_{29}H_{35}N_5O_6$: 549.6. Found $(M+Na)^+$: 572.3.

Example D

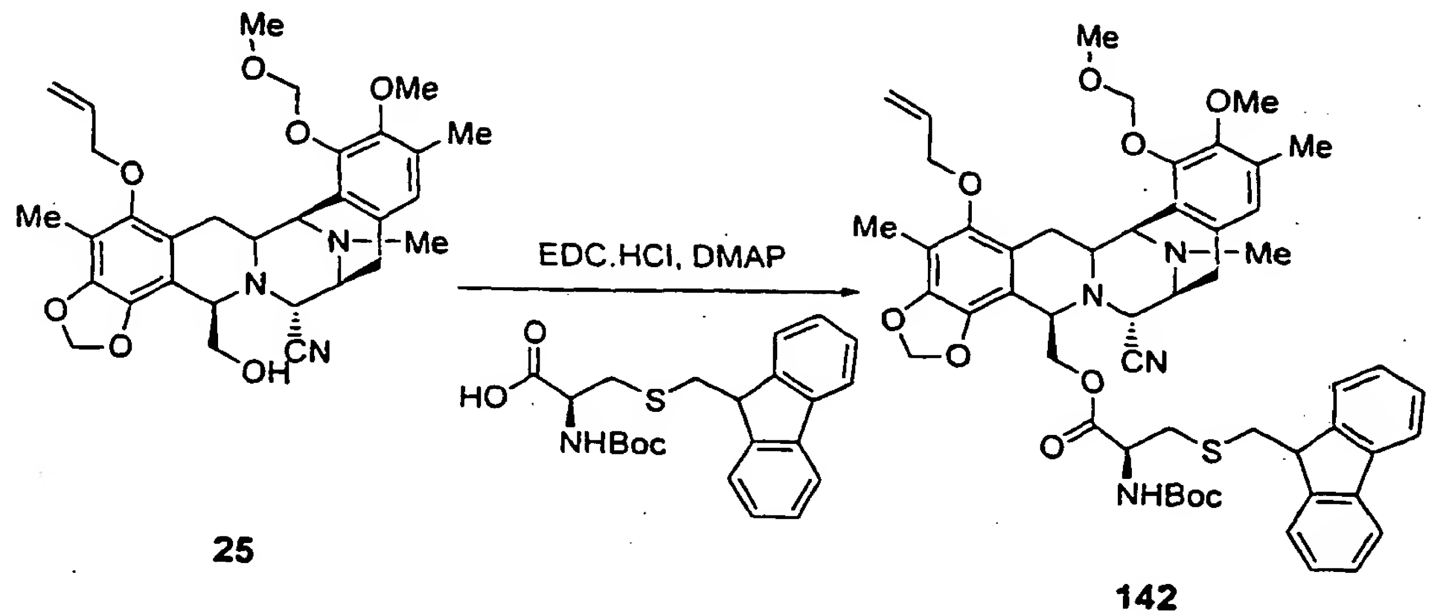
A medium (50 l) composed of dextrose (2%), mannitol (4%), dry brewer's yeast (2%), ammonium sulphate (1%), potassium secondary phosphate (0.04%), potassium chloride (0.8%), iron (III) chloride 6-hydrate (0.001%), L-tyrosine (0.1%), calcium carbonate (0.8%), poly-(propylene glycol) 2000 (0.05%) and antifoam ASSAF 1000 (0.2%) was poured into a jar-fermentor with 75 l total capacity and, after sterilisation, inoculated with seed culture (2%) of A2-2 strain (FERM BP-14) and aerated cultivation under agitation was carried out at 27°C to 24°C for 64 hours (aeration of 75 l per minute and agitation from 350 to 500 rpm). The pH was controlled by automatic feeding of diluted sulphuric acid from 27 hours to final process. A 2% mannitol was added from 16 hours to final process. The cultured medium (45 l) thus obtained was, after removal of cells by centrifugation, adjusted to pH 9.5 with diluted sodium hydroxide, extracted with 25 litres of ethyl acetate twice. The mixture was carried out into an agitated-vessel at 8°C for 20 minutes. The two phases were separated by a liquid-liquid centrifuge. The organic phases were frozen at -20°C and filtered for removing ice and evaporated ice and evaporated until obtention of a 40 g oil-dark-crude extract. After introduction of the cyanide group and purification, 3.0 grams of safracin B cyano were obtained.

Example E

A medium (50 l) composed of dextrose (2%), mannitol (4%), dry brewer's yeast (2%), ammonium sulphate (1%), potassium secondary phosphate (0.02%, potassium chloride (0.2%), Iron (III) chloride 6-hydrate (0.001%, L-tyrosine (0.1%), calcium carbonate (0.8%, poly-(propylene glycol) 2000 (0.05%) and antifoam ASSAF 1000 (0.2%) was poured into a jar-fermentor with 75 l total capacity and, after sterilisation, inoculated with seed culture (2%) of A2-2 strain (FERM BP-14) and aerated cultivation under agitation was carried out at 27°C to 24°C for 41 hours (aeration of 75 l per minute and agitation from 350 to 500 rpm). The pH was controlled by automatic feeding of diluted sulphuric acid from 28 hours to final process. A 1% mannitol was added from 16 hours to final process. The cultured medium (45 l) thus obtained was, after removal of cells by centrifugation, adjusted to pH 3.9 with 200 ml of conc. acetic acid. 25 grams of potassium cyanide 97% were added and after 1 hour of agitation at 20°C, the pH was adjusted to 9.5 with 1500 ml of a solution 10% sodium hydroxide. Then, extracted with 35 litres of ethyl acetate. The mixture was carried out into an agitated -vessel at 8°C for 20 minutes. The two phases were separated by a liquid-liquid centrifuge. The organic phase was dried by sodium sulphate anhydrous and evaporated until obtention of a 60 g oil-dark-crude extract.

After chromatography, 4.9 grams of safracin B cyano were obtained.

Example 66

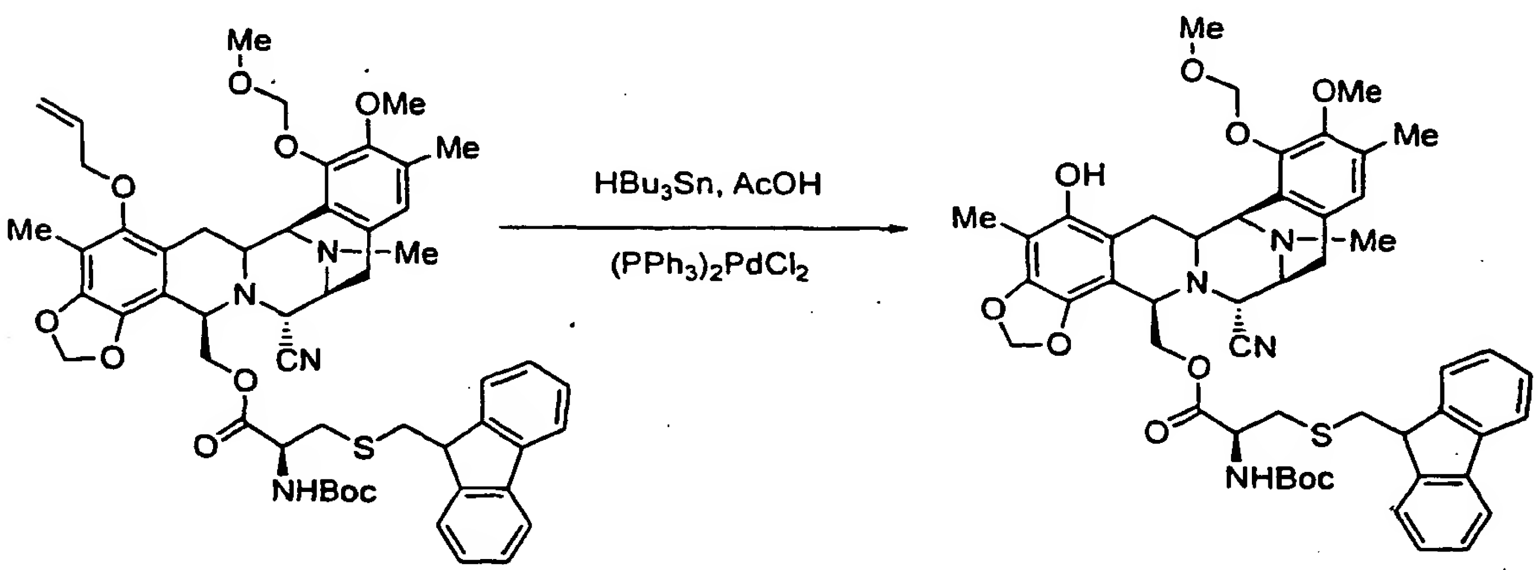


To a stirred solution of **25** (7.83 g, 0.0139 mol) and the commercial available Boc-Cys (Fm) derivative (Bachem) (8.33 g, 35.04 mmol) in dichloromethane (535 mL) under argon, dimethylaminopyridine (4.28 g, 35.04 mmol) and 1-[3-(Dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride (6.66 g, 35.04 mmol) were added at 23 °C. The mixture was then stirred at 23 °C for 2.5 hours. The reaction was quenched by addition of a saturated aqueous sodium bicarbonate solution (500 mL), the organic phase separated and the aqueous layer back-extracted with dichloromethane (250 mL). The combined organic extracts were dried over sodium sulphate, filtrated and evaporated to dryness under reduced pressure. The crude product was purified by flash column chromatography eluting with mixtures of ethyl acetate and hexane in a gradient manner, from 1:4 to 2:1 to yield **142** (12.21 g, 93%) as a light yellow solid. Rf = 0.35 Hex:EtOAc 1:1.

^{1}H-NMR (300 MHz, $CDCl_3$): δ 7.72 (d, J=7.3, 2.7 Hz 2H), 7.55 (dd, J_1 = 14.6, J_2 = 7.6 2H), 7.40-7.34 (m, 2H), 7.30-7.24 (m, 2H), 6.63 (s, 1H), 6.08-5.99 (m, 1H), 5.91 (d, J= 1.5 Hz, 1H), 5.80 (d, J= 1.5 Hz, 1H), 5.39 (dd, J_1 = 17.3, J_2 = 1.7 Hz 1H), 5.24 (dd, J_1 = 10.5, J_2 = 1.7 Hz, 1H), 5.09 (AB, J = 4.48 Hz, 2H), 5.07 (t, J = 7.8 Hz, 1H), 4.34-4.29 (m, 2H), 4.17 (d, J = 1.9 Hz, 1H), 4.16-4.04 (m, 4H), 4.02-3.96 (m, 2H), 3.93 (t, J

= 5.3 Hz, 1H), 3.70 (s, 3H), 3.56 (s, 3H), 3.32 (d, J = 8.0, 1H), 3.23-3.17 (m, 2H), 3.0-2.89 (m, 3H), 2.65-2.57 (m, 2H), 2.29 (s, 3H), 2.20 (s, 3H), 2.03 (s, 3H), 1.76 (dd, J_1 = 16.3, J_2 = 12.7 Hz, 1H), 1.45, 1.44 (s, 9H).
^{13}C-NMR (75 MHz, $CDCl_3$): δ 170.9, 155.3, 148.9, 148.6, 146.1, 146.0, 144.7, 141.2, 141.1, 139.4, 134.0, 131.0, 130.1, 127.8, 127.2, 125.2, 125.0, 124.3, 121.3, 121.2, 120.1, 118.1, 117.6, 112.9, 101.4, 99.5, 80.3, 74.2, 65.6, 60.4, 60.1, 57.9, 57.4, 57.2, 57.1, 56.9, 55.6, 53.2, 47.0, 41.8, 41.7, 36.7, 35.3, 28.5, 26.6, 25.3, 15.9, 9.4.
ESI-MS m/z: Calcd. For $C_{53}H_{60}N_4O_{10}S$: 945.13. Found $(M+1)^+$: 946.3.

Example 67



142 143

To a stirred solution of **142** (12.01 g, 0.0127 mol) in dichloromethane (318 mL), dichlorobis(triphenylphosphine) palladium (II) (0.71g, 1.015 mmol) and acetic acid (3.6 mL, 0.176 mol) were added under argon at 23 °C. Then, tributyl tin hydride (10.27 mL, 0.037 mol) was added in a dropwise manner. The mixture was stirred at 23 °C for 10 minutes. The reaction was then filtered through a silica gel column compacted with hexane. **143** (10.89 g, 95%) was obtained as a yellow solid by

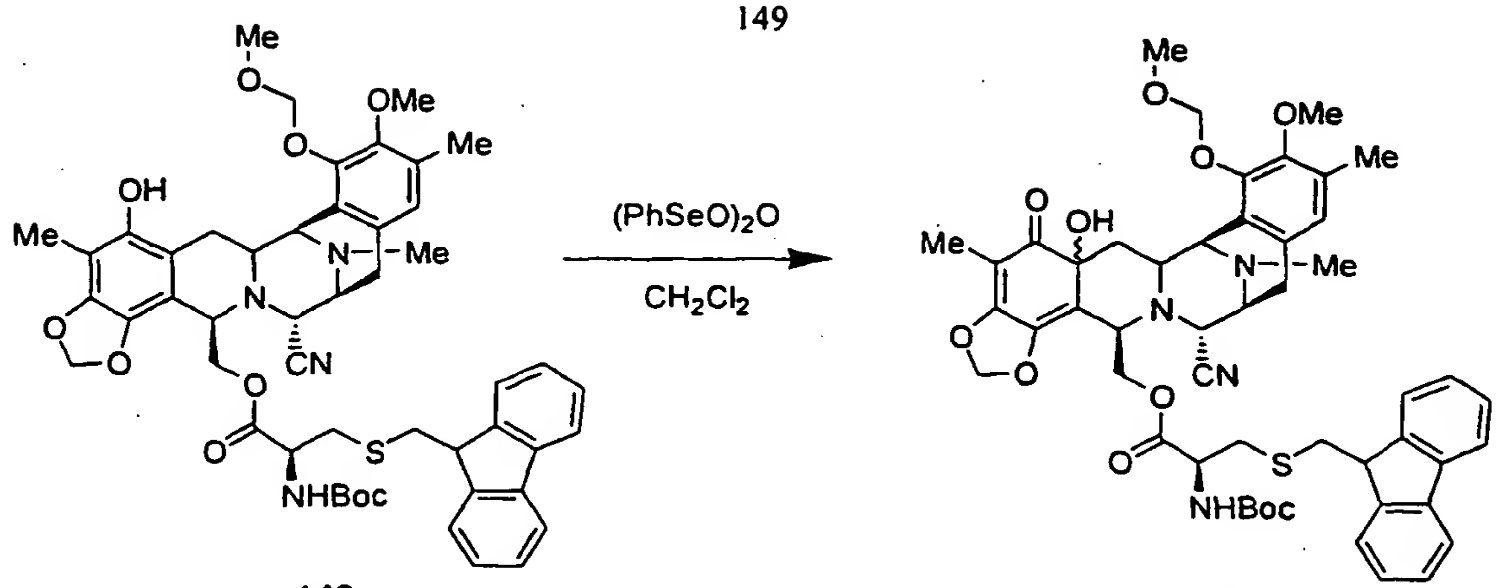
subsequent elution with mixtures of ethyl acetate and hexane in a gradient manner, from 1:4, 1:1 to 7:3. Rf = 0.25 Hex:EtOAc 2:1.

^1H NMR (300 MHz, $CDCl_3$) δ 7.72 (d, J = 7.6 Hz, 2H), 7.61 (d, J = 6.6 Hz, 1H), 7.52 (d, J = 7.3 Hz, 1H), 7.37 (t, J = 7.8 Hz, 2H), 7.28 (m, 2H), 6.63 (s, 1H), 5.87 (d, J = 1.5 Hz, 1H), 5.76 (d, J = 1.5 Hz, 1H), 5.58 (bs, 1H), 5.31 (d, J = 5.8 Hz, 1H), 5.17 (d, J = 5.6 Hz, 1H), 4.91 (d, J = 8.3Hz, 1H), 4.17-4.06 (m, 4-6H), 3.85 (t, J = 5.7 Hz, 1H), 3.70 (s, 3H), 3.68 (s, 3H), 3.34 (brd, J = 6.6 Hz, 1H), 3.23 (brd, J = 11.2 Hz, 1H), 3.06 (brd, J = 12.9 Hz, 1H), 3.04-2.86 (m, 3H), 2.65-2.54 (m, 2H), 2.28 (s, 3H), 2.21 (s, 3H), 1.94 (s, 3H), 1.80 (dd, J_1 = 11.5 Hz, J_2 = 15.8 Hz, 1H), 1.45 (s, 9H).

^{13}C NMR (75 MHz, $CDCl_3$) δ175.3, 170.5, 154.9, 149.1, 147.6, 145.9, 145.8, 145.7, 144.5, 140.9, 140.8, 136.1, 130.9, 127.4, 126.9, 124.3, 124.7, 122.9, 119.7, 117.6, 112.3, 111.4, 106.6, 100.7, 99.7, 80.0, 64.2, 60.3, 59.8, 57.6, 57.0, 56.5, 56.4, 55.2, 52.7, 46.7, 46.5, 41.4, 41.3, 36.9, 36.6, 34.9, 28.2, 26.0, 24.9, 20.9, 20.7, 15.7, 14.1, 8.5.

ESI-MS m/z: Calcd. For $C_{50}H_{56}N_4O_{10}S$: 905.5. Found $(M+1)^+$: 906.3.

Example 68



To a solution of **143** (10 g, 0.011 mol) in anhydrous dichloromethane (185 mL) at −10 °C (bath temperature −15 °C), a solution of benzeneseleninic anhydride (5.7 g, 0.011 mol) was added in anhydrous dichloromethane (185 mL), discarding any white solid present in the solution. The mixture was stirred for 10 minutes at the same temperature. The reaction was diluted with dichloromethane (200 mL) and a saturated aqueous sodium bicarbonate solution (500 mL) was added at −10 °C. The organic phase was separated, dried over sodium sulphate, filtered and concentrated to dryness at reduced pressure. The residue was purified by flash column chromatography, eluting with mixtures of ethyl acetate and hexane in a gradient manner, from 1:1, 3:2, 7:3 to 4:1 to obtain **144** (9.34 g, 92%) as a yellow solid. The purified solid from chromatography was dissolved in dichloromethane (250 mL), charcoal (3.3 g) was added and the suspension was stirred at 23 °C for 1 hour. The mixture was filtered through celite and the celite was washed with dichloromethane (80 mL). The solvent was evaporated at reduced pressure maintaining the temperature at 25-30 °C to yield **144** (8.96 g, 88%) as a yellow solid. Rf = 0.30 and 0.25 (mixture of isomers) Hex:EtOAc 1:1.

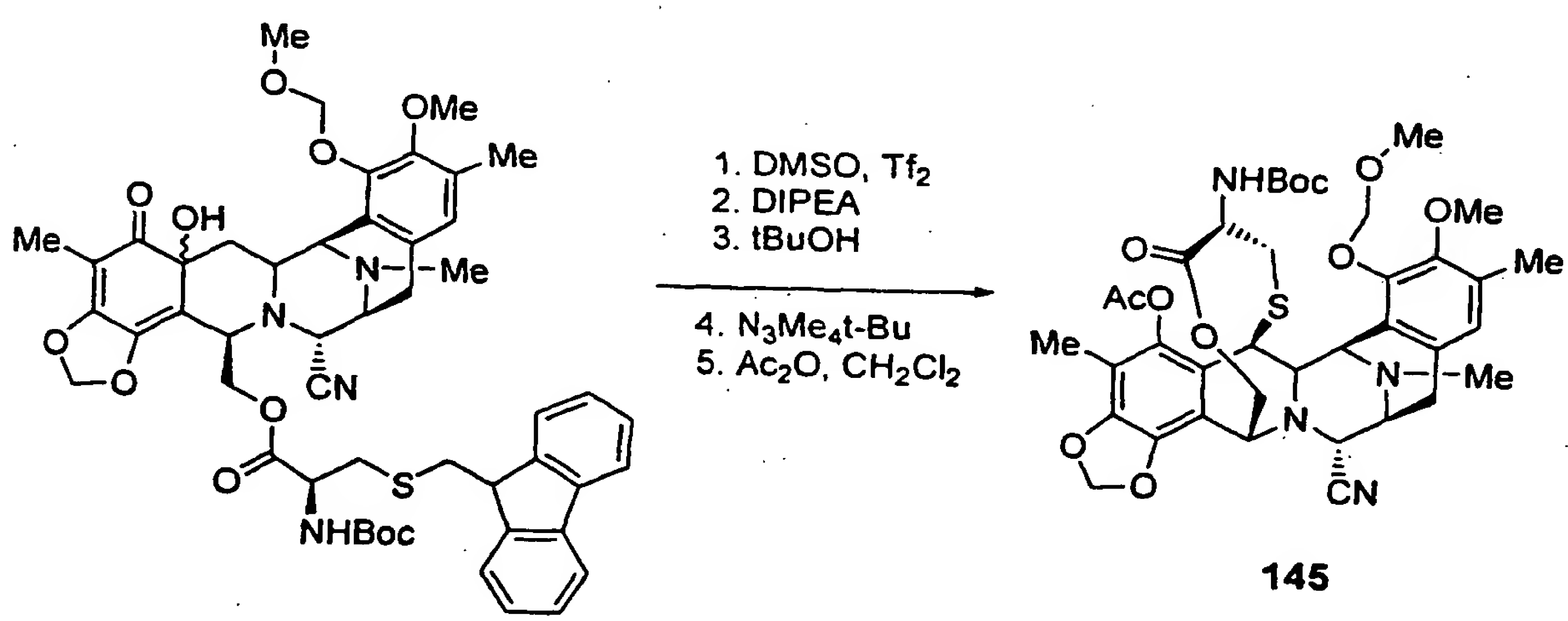
^1H NMR (300 MHz, $CDCl_3$) (mixture of isomers) δ 7.73-7.61 (m, 4H), 7.37-7.30 (m, 4H), 6.62 (s, 1H), 6.59 (s, 1H), 6.53 (s, 1H), 5.72 (s, 1H), 5.70 (s, 1H), 5.61 (s, 1H), 5.55 (bs, 1H), 5.34 (m, 2H), 5.08 (AB sist., J_{AB}

= 6.7 Hz, 1H), 5.00 (AB sist., J_{AB} = 5.9 Hz, 1H), 4.67 (m, 1H), 4.50 (m, 1H), 4.38 (dd, J_1 = 4.9 Hz, J_2 = 12.9 Hz, 1H), 4.21 (dd, J_1 = 6.3 Hz, J_2 = 12.9 Hz, 1H), 4.11 (t, J = 5.9 Hz, 1H), 4.02 (m, 3H), 3.87 (m, 1H), 3.83 (s, 3H), 3.72 (m, 1H), 3.61 (s, 3H), 3.49 (s, 3H), 3.27 (m, 1H), 3.15 (dd, J_1 = 1.8 Hz, J_2 = 6.2 Hz, 2H), 3.07 (d, J = 6.3 Hz, 1H), 2.94 (m, 4H), 2.86 (m, 2H), 2.42 (m, 2H), 2.25 (s, 3H), 2.20 (s, 3H), 2.15 (s, 3H), 2.08 (dd, J_1 = 2.4 Hz, J_2 = 13.9 Hz, 1H), 1.77 (s, 3H), 1.76 (s, 3H), 1.43 (s, 9H).

^{13}C NMR (75 MHz, $CDCl_3$) (mixture of isomers) δ 200.6, 171.2, 160.4, 155.6, 148.9, 148.8, 148.3, 145.9, 145.8, 141.3, 141.2, 138.7, 130.9, 127.9, 127.4, 127.3, 127.3, 125.3, 125.1, 124.2, 120.1, 117.1, 111.9, 108.5, 105.0, 104.7, 101.7, 101.3, 99.5, 99.4, 80.5, 72.5, 70.8, 60.5, 60.1, 58.4, 58.0, 57.9, 56.9, 56.8, 56.3, 55.9, 55.5, 55.4, 53.8, 53.7, 47.1, 42.0, 41.8, 41.5, 37.4, 37.3, 35.6, 35.5, 28.5, 25.8, 25.7, 16.1, 16.0, 7.7, 7.3.

ESI-MS m/z: Calcd. for $C_{50}H_{56}N_4O_{11}S$: 921.3. Found $(M+1)^+$: 922.3.

Example 69



144

145

To a solution of DMSO (3.44 mL) in anhydrous dichloromethane (396 mL), triflic anhydride (3.27 mL, 19.45 mmol) was added under argon at –78 °C and the mixture was stirred at –that temperature for 20 minutes. Then, a solution of **144** (8.92 g, 9.6 mmol) in anhydrous

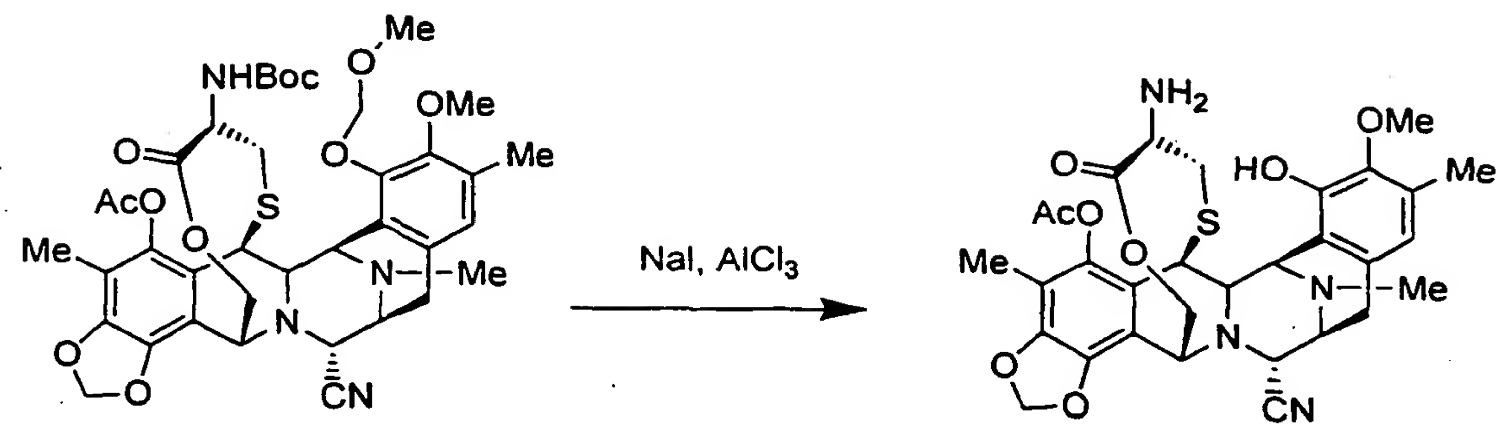
dichloromethane (124 mL) at –78 °C was added and the mixture was stirred under argon at –40 °C for 35 minutes. Diisopropylethylamine (13.5 mL, 73.43 mmol) was added and the mixture was stirred under argon for 45 minutes at 0 °C. *Tert*-butanol (3.65 mL, 38.6 mmol) and *tert*-butyl tetramethyl guanidine (11.6 mL, 67.46 mmol) were added and the mixture was stirred under argon for 40 minutes at 23 °C. Acetic anhydride (9.15 mL, 96.78 mmol) was then added and the reaction stirred for a further 1 hour at 23 °C. The reaction was diluted with dichloromethane (250 mL) and a saturated aqueous ammonium chloride solution (500 mL) was added. The organic layer was separated and washed sequentially with a saturated aqueous sodium bicarbonate solution (500 mL) and a saturated aqueous sodium chloride solution (500 mL). The organic layer was separated, dried over sodium sulphate, filtered and concentrated to dryness at reduced pressure, maintaining the temperature at 25-30 °C. The crude solid was then purified by flash column chromatography, eluting with mixtures of ethyl acetate and hexane in a gradient manner, from 1:4 to 2:3 to give **145** (4.99 g, 68%) as a yellow solid. Rf = 0.44 Hex:EtOAc 3:2.

^{1}H NMR (300 MHz, $CDCl_3$) (mixture of isomers) δ 6.79 (s, 1H), 6.09 (s, 1H), 6.00 (s, 1H), 5.20 (d, J = 5.4 Hz, 1H), 5.14 (d, J = 5.6 Hz, 1H), 5.02 (d, J = 11.7 Hz, 1H), 4.63 (d, J = 9.0 Hz, 1H), 4.50 (m, 1H), 4.33 (d, J = 5.4 Hz, 1H), 4.30 (m, 1H), 4.25 (bs, 1H), 4.18 (d, J = 2.4Hz, 1H), 4.17 (dd, J_1 = 1.3 Hz, J_2 = 11.7 Hz, 1H), 3.78 (s, 3H), 3.57 (s, 3H), 3.42 (m, 2H), 2.93 (m, 2H), 2.35 (m, 1H), 2.31 (s, 3H), 2.29 (s, 3H), 2.22 (s, 3H), 2.09 (m, 1H), 2.05 (s, 3H), 1.45 (s, 9H).).

^{13}C NMR (75 MHz, $CDCl_3$) δ 207.3, 170.9, 168.8, 155.4, 149.8, 148.6, 146.0, 141.1, 140.7, 131.7, 130.6, 125.1, 120.6, 118.3, 113.7, 102.2, 99.4, 80.0, 61.6, 60.4, 59.8, 59.4, 59.2, 57.7, 55.0, 54.7, 54.0, 41.9, 41.6, 33.1, 31.8, 28.7, 23.9, 20.6, 16.1, 14.3, 9.8.

ESI-MS m/z: Calcd. for $C_{38}H_{46}N_4O_{11}S$: 766.86. Found $(M+1)^+$: 767.3.

Example 70



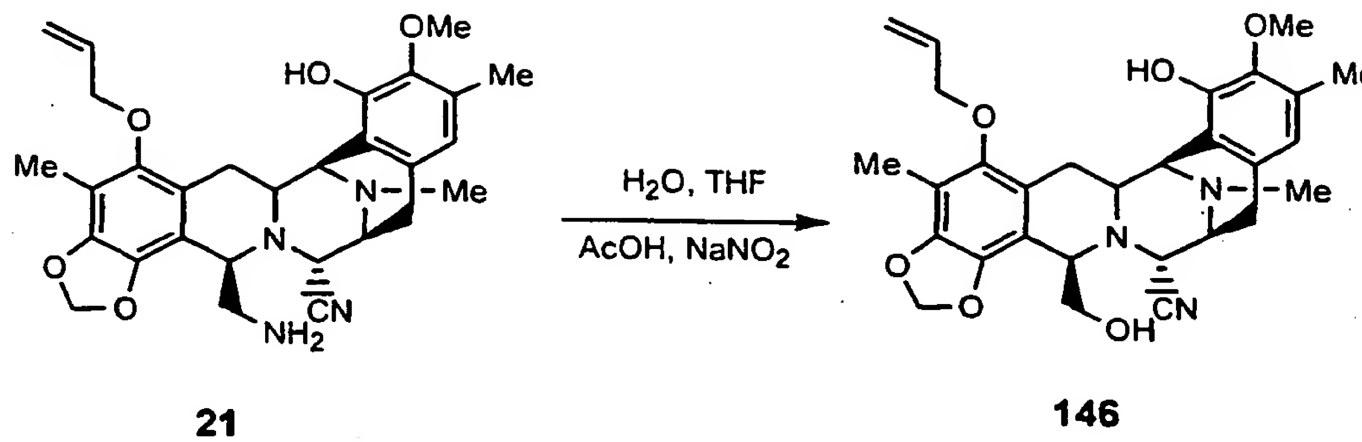
145 35

To a solution of **145** (1.0 g, 1.3 mmol) in acetonitrile (50 mL) and dichloromethane (25 mL), sodium iodide (1.52 g, 10.01 mmol) was added at 23 °C. The mixture was then cooled to 0 °C and aluminium trichloride (1.33 g, 10.01 mmol) was added portionwise maintaining the temperature at 0 °C. The mixture was then stirred for 2.5 hours at 0 °C. The reaction was diluted with dichloromethane (25 mL) and a saturated aqueous solution of sodium potassium tartrate (100 mL) was added. The aqueous phase is separated and extracted with dichloromethane (2 x 75 mL). A saturated aqueous sodium bicarbonate solution (50 mL) was then added to the aqueous phase which was further extracted with dichloromethane (2 x 50 mL). The combined organic extracts were dried over sodium sulphate, filtered and evaporated to dryness under reduced pressure, maintaining the temperature below 25 °C. The crude solid was then purified by column chromatography on amino-silicagel and eluting with mixtures of ethyl acetate and hexane in a gradient manner. **35** (487 mg, 60%) was obtained as a yellow solid. Experimental data of **35** were previously described in PCT/GB00/01852.

36, ET-770 and **ET-743** were prepared following the same procedures than those previously described in PCT/GB00/01852.

Route 2

Example 71



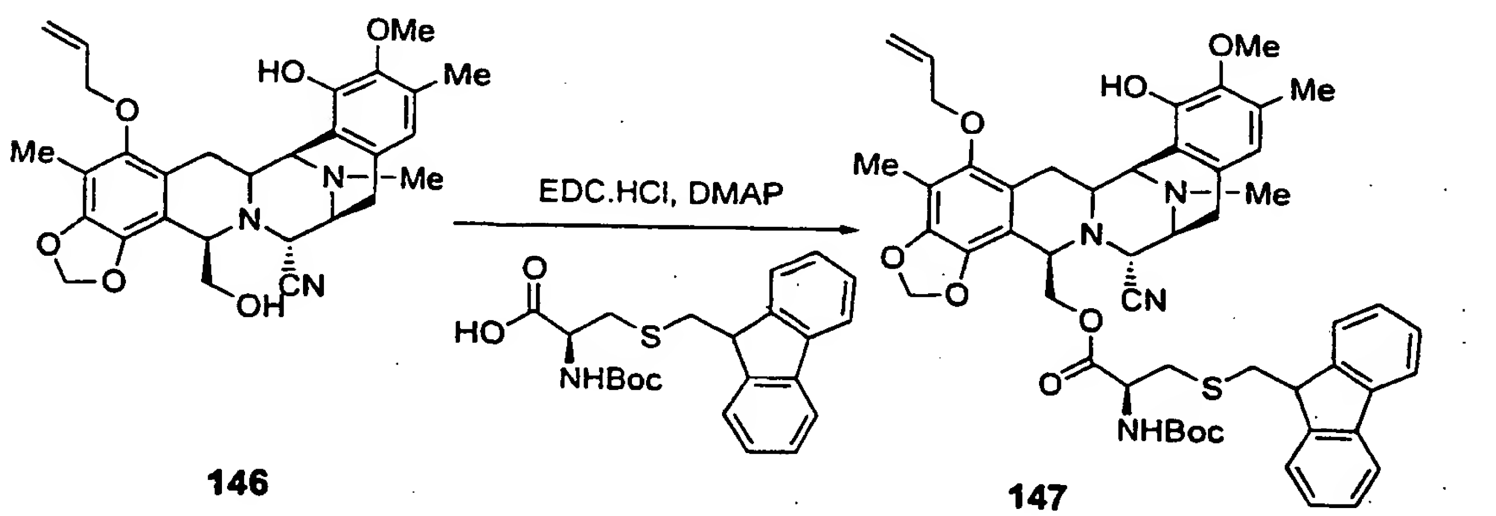
A solution of **21** (9.84 g, 18.97 mmol) in THF (569 mL) and H_2O (285 mL) was cooled at 0°C with an ice bath. Then, $NaNO_2$ (1.96 g, 28.45 mmol) and 90% aq. AcOH (18.97 mL, 0.33 mol) were added at 0 °C and the mixture was stirred at 23 °C for 18h. After cooling down the reaction to 0°C, a saturated aqueous sodium bicarbonate solution (300 mL, basic pH) and dichloromethane (500 mL) were added. After extraction, the aqueous phase was further extracted with dichloromethane (2 x 300 mL). The combined organic extracts were dried over sodium sulphate and evaporated to dryness under reduced pressure. The crude solid was then disolved in MeOH (379 mL), and 1M NaOH (38 mL) was added at 0°C. The mixture was stirred at 23 °C for 4h. After dilution with EtOAc (600 mL) at 0°C, the organic layer was washed with a mixture of water (400 mL) and , a saturated aqueous sodium bicarbonate solution (100 mL, basic pH). After extraction, the aqueous phase was further extracted with EtOAc (3 x 300 mL). The combined organic extracts were dried over Na_2SO_4, filtered and concentrated *in vacuo*. The residue was purified by flash column chromatography (SiO_2, Hex:EtOAc gradient from 3:1 to 2:1) to afford **146** (4.55 g, 46%) as a white solid. Rf: 0.33 (Hex:EtOAc 1:1).

^1H NMR (300 MHz, $CDCl_3$) δ 6.48 (s, 1H), 6.15-6.02 (m, 1H), 5.92 (d, J = 1.5 Hz, 1H), 5.86 (d, J = 1.5 Hz, 1H), 5.77 (s, 1H), 5.39 (dd, J_1 = 1.5 Hz, J_2 = 17.1 Hz, 1H), 5.26 (dd, J_1 = 1.5 Hz, J_2 = 10.5 Hz, 1H), 4.24-4.15 (m, 3H), 4.04 (d, J = 2.4 Hz, 1H), 3.97 (t, J = 3.3 Hz, 1H), 3.74 (s, 3H), 3.64 (dt, , J_1 = 3.3 Hz, , J_2 = 11.1 Hz, 1H), 3.43 (dd, J_1 = 3.3 Hz, J_2 = 10.5 Hz, 1H), 3.38-3.34 (m, 2H), 3.31 (t, J = 2.7 Hz, 1H), 3.22 (dd, J_1 = 2.4 Hz, J_2 = 15.6 Hz, 1H), 3.10 (dd, J_1 = 8.1 Hz, , J_2 = 18.3 Hz, 1H), 2.49 (d, J = 18.3 Hz, 1H), 2.34 (s, 3H), 2.24 (s, 3H), 2.11 (s, 3H), 1.88 (dd, J_1 = 12 Hz, J_2 = 15.9 Hz, 1H).

^{13}C NMR (75 MHz, $CDCl_3$) δ 148.6, 146.7, 144.4, 143.0, 138.9, 133.9, 130.2, 129.1, 121.1, 120.9, 117.7, 117.4, 116.8, 113.3, 112.3, 101.1, 74.3, 63.7, 60.6, 60.1, 58.1, 56.9, 56.7, 55.4, 41.7, 26.2, 25.7, 15.7, 9.3.

ESI-MS m/z: Calcd. for $C_{29}H_{33}N_3O_6$: 519.59. Found $(M+1)^+$: 520.5.

Example 72



To a stirred solution of **146** (47.35 g, 0.091 mol) and the commercial available Boc-Cys (Fm) derivative (54.6 g, 0.137 mol) in dichloromethane (2.8 L) under argon, dimethylaminopyridine (5.6 g, 0.046 mol) and 1-[3-(Dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride (43.6 g, 0.227 mol) were added dropwise during 1.5 h at 23 °C. The mixture was then stirred at 23 °C for 1 more hour. The reaction was quenched by addition of a saturated aqueous sodium

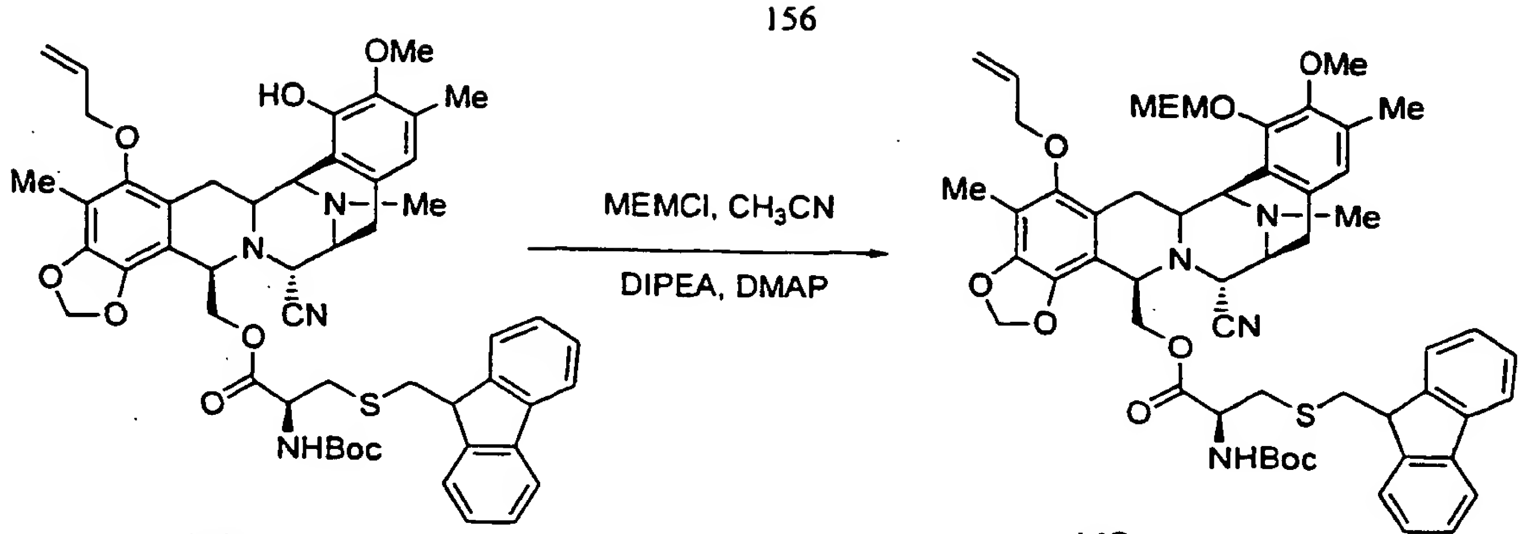
bicarbonate solution (1 L) and the organic phase was separated. The aqueous layer was back-extracted with dichloromethane (2 x 500 mL). The combined organic extracts were dried over sodium sulphate and evaporated to dryness under reduced pressure. The crude product was purified by flash column chromatography eluting with mixtures of ethyl acetate and hexane in a gradient manner, from 1:4 to 3:1 to yield **147** (74.3 g, 93%) as a white solid. Rf = 0.5 Hex: EtOAc 1:1.

^{1}H NMR (300 MHz, $CDCl_3$) δ 7.73 (d, J = 7.8 Hz, 2H), 7.63-7.55 (m, 2H), 7.39-7.35 (m, 2H), 7.29-7.25 (m, 2H), 6.41 (s, 1H), 6.07-5.97 (m, 1H), 5.92 (d, J = 1.2 Hz, 1H), 5.80 (d, J = 1.2 Hz, 1H), 5.67 (s, 1H), 5.34 (dd, J_1 = 1.8 Hz, J_2 = 17.4 Hz, 1H), 5.23 (dd, J_1 = 1.8 Hz, J_2 = 10.5 Hz, 1H), 5.04 (d, J = 9.3 Hz, 1H), 4.32-4.29 (m, 1H), 4.13-3.91 (m, 9H), 3.72 (s, 3H), 3.31 (d, J = 7.2 Hz, 1H), 3.26-3.17 (m, 2H), 2.96-2.87 (m, 3H), 2.68-2.54 (m, 2H), 2.27 (s, 3H), 2.24 (s, 3H), 2.05 (s, 3H), 1.83 (dd, J_1 = 12.6 Hz, J_2 = 15.9 Hz, 1H), 1.45 (s, 9H).

^{13}C NMR (75 MHz, $CDCl_3$) δ 170.9, 155.4, 149.0, 147.1, 146.2, 146.0, 144.7, 143.0, 141.1, 139.4, 134.1, 131.5, 129.1, 127.8, 127.2, 125.0, 121.3, 120.9, 120.1, 118.2, 117.6, 117.2, 112.9, 112.4, 101.4, 80.3, 76.6, 74.4, 65.3, 61.0, 60.4, 57.4, 56.9, 56.7, 55.6, 53.0, 46.9, 41.8, 36.7, 35.3, 31.8, 28.5, 26.6, 25.2, 22.9, 16.0, 14.4, 9.5.

ESI-MS m/z: Calcd. for $C_{51}H_{56}N_4O_9S$: 900.3. Found $(M+1)^+$: 901.3.

Example 73



147 **148**

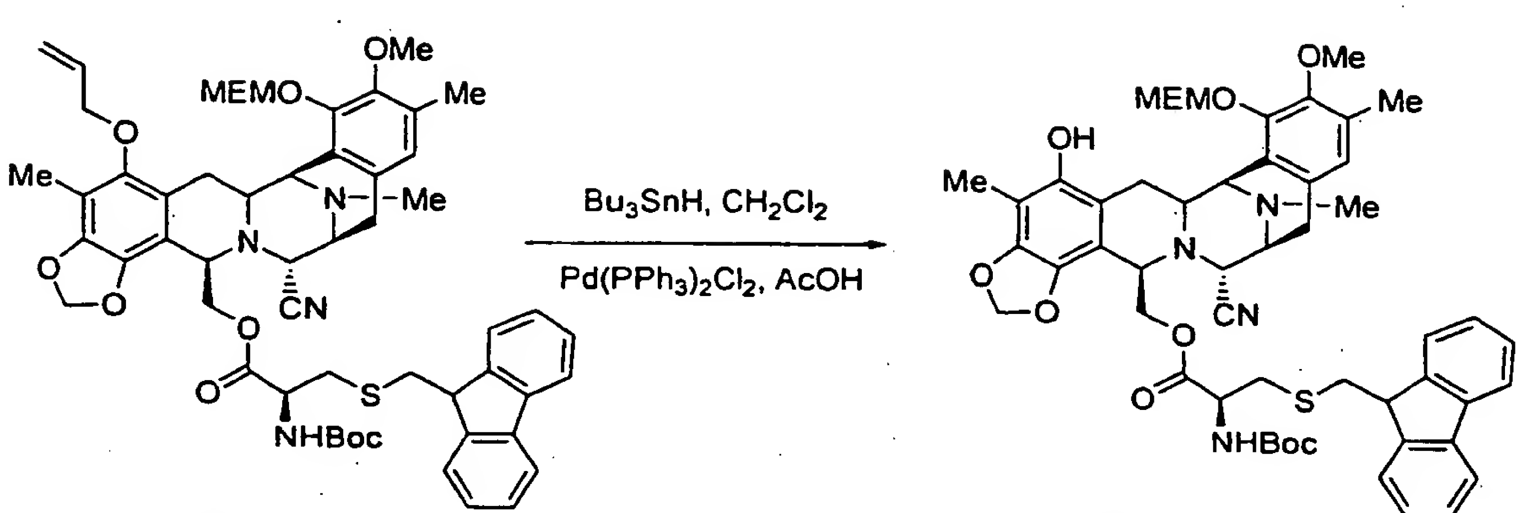
To a solution of **147** (0.562 g, 0.624 mol) in CH_3CN (3.12 mL), MEMCl (1.07 mL, 9.36 mmol), DIPEA (2.17 mL, 12.48 mmol) and DMAP (0.0076 g, 0.06 mmol) were added at 0°C. The mixture was stirred for 5.5 h at 23°C. The reaction was diluted with CH_2Cl_2 (50 mL) and extracted with 0.1N HCl (50 mL). The aqueous phase was extracted again with CH_2Cl_2 (50 mL). The combined organic phases were dried over Na_2SO_4, filtered and concentrated *in vacuo* to give a residue which was purified by flash column chromatography (CH_2Cl_2:EtOAc 10:1, 5:1) to give **148** (539 mg, 87%) as a white solid. Rf= 0.50 CH_2Cl_2:AcOEt 6:1.

^1H NMR (300 MHz, $CDCl_3$) δ 7.73-7.71 (m, 2H), 7.57 (dd, J_1 = 7.2 Hz, J_2 = 15.3 Hz, 2H), 7.40-7.34 (m, 2H), 7.29-7.26 (m, 2H), 6.62 (s, 1H), 6.08-5.99 (m, 1H), 5.91 (d, J = 1.2 Hz, 1H), 5.79 (d, J = 1.2 Hz, 1H), 5.35 (dd, J_1 = 1.2 Hz, J_2 = 17.1 Hz, 1H), 5.23 (d, J = 6.3 Hz, 1H), 5.21 (bs, 1H), 5.13 (d, J = 6.3 Hz, 1H), 5.04 (brd, J = 9 Hz, 1H), 4.33-4.29 (m, 2H), 4.16-3.90 (m, 8H), 3.85-3.78 (m, 1H), 3.69 (s, 3H), 3.60-3.55 (m, 2H), 3.38 (s, 3H), 3.31 (brd, J = 8.1 Hz, 1H), 3.21-3.17 (m, 2H), 2.98-2.88 (m, 3H), 2.64-2.56 (m, 2H), 2.29 (s, 3H), 2.20 (s, 3H), 2.02 (s, 3H), 1.75 (dd, J_1 = 11.7 Hz, J_2 = 15.6 Hz, 1H), 1.47 (s, 9H).

^{13}C NMR (75 MHz, $CDCl_3$) δ 170.5, 155.0, 148.6, 148.5, 148.2, 145.77, 145.6, 144.4, 140.8, 140.7, 139.0, 133.6, 130.7, 130.5, 127.4, 126.9, 124.8, 124.6, 123.8, 120.8, 119.7, 117.8, 117.2, 122.5, 111.9, 101.0, 98.1, 80.0, 77.4, 77.0, 76.6, 73.8, 71.6, 69.2, 65.0, 60.2, 60.0, 59.8, 59.0, 56.8, 56.7, 56.6, 55.2, 52.7, 46.6, 41.3, 36.2, 34.9, 29.6, 28.2, 26.3, 24.9, 15.6, 14.1, 9.0.

ESI-MS m/z: Calcd. for $C_{55}H_{64}N_4O_{11}S$: 988.4. Found $(M+1)^+$: 989.3.

Example 74



148 149

To a stirred solution of **148** (38.32 g. 0.039 mol) in dichloromethane (1 L), dichlorobis(triphenylphosphine) palladium (II) (2.17 g, 0.0031 mol) and acetic acid (11.1 mL, 0.195 mol) were added under argon at 23 °C. Then, tributyl tin hydride (36.5 mL, 0.136 mol) was added in a dropwise manner. The mixture was stirred at 23 °C for 15 minutes. The reaction was then filtered through a silica gel column compacted with hexane. **149** (35.07 g, 95%) was obtained as a white solid by subsequent elution with mixtures of ethyl acetate and hexane in a gradient manner, from 0:100, 1:4, 1:3, 2:5, 2:3, 1:1, 2:1, 3:1 to 100:0. Rf = 0.25 Hex:EtOAc 2:1.

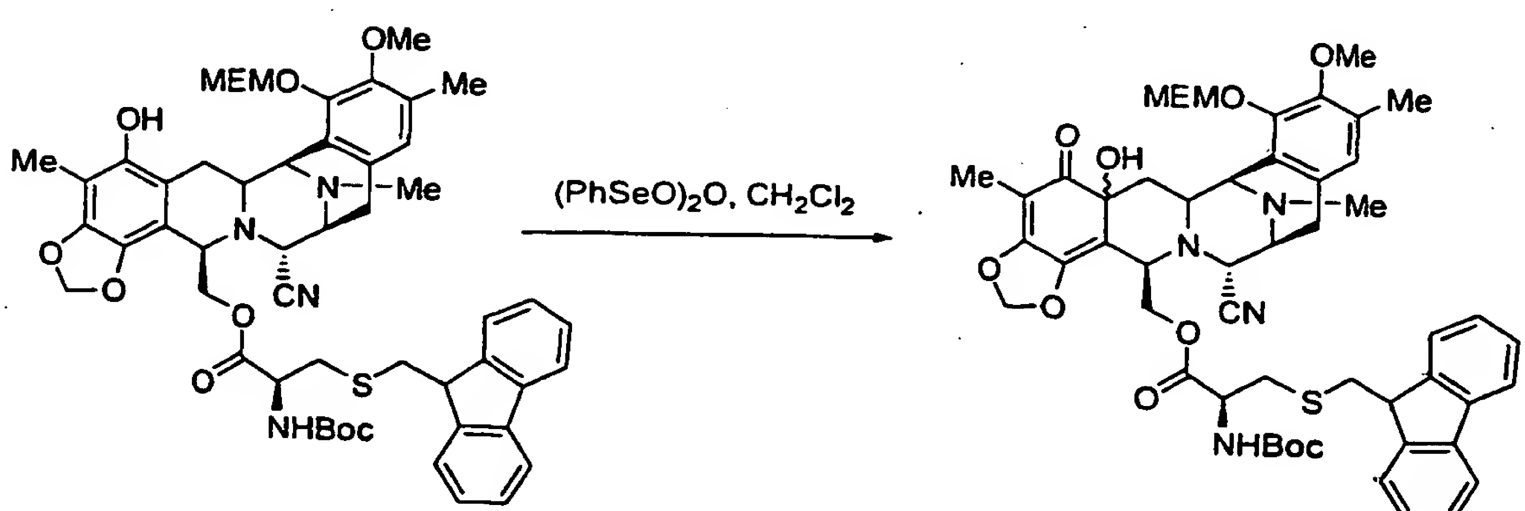
^1H NMR (300 MHz, $CDCl_3$) δ 7.74 (d, J = 7.2 Hz, 2H), 7.63-7.53 (m, 2H), 7.39-7.34 (m, 2H), 7.30-7.27 (m, 2H), 6.62 (s, 1H), 5.87 (m, 1H), 5.75 (s, 1H), 5.69 (bs, 1H), 5.37 (d, J = 6 Hz, 1H), 5.23 (d, J = 5.7 Hz, 1H), 4.96

(d, J = 8.1 Hz, 1H), 4.44 (brd, J = 8.7 Hz, 1H), 4.18-3.70 (m, 11H), 3.69 (s, 3H), 3.38 (s, 3H), 3.34-3.18 (m, 3H), 2.99-2.88 (m, 3H), 2.63-2.58 (m, 2H), 2.28 (s, 3H), 2.21 (s, 3H), 2.05 (s, 3H), 1.78 (dd, J_1 = 12.9 Hz, J_2 =15.6.3 Hz, 1H), 1.41 (s, 9H).

^{13}C NMR (75 MHz, $CDCl_3$) δ 170.8, 155.2, 149.0, 148.0, 146.2, 146.0, 144.8, 141.1, 136.4, 131.3, 131.2, 127.8, 127.2, 125.1, 125.0, 123.2, 120.0, 118.1, 112.6, 111.6, 107.2, 101.0, 98.9, 98.8, 80.3, 71.8, 69.8, 64.9, 60.6, 60.2, 59.2, 57.1, 56.9, 55.5, 53.0, 47.0, 46.9, 41.8, 37.0, 35.3, 28.5, 26.2, 25.2, 21.9, 21.3, 16.1, 14.4, 9.0.

ESI-MS m/z: Calcd. for $C_{52}H_{60}N_4O_{11}S$: 948.4. Found $(M+1)^+$: 949.3.

Example 75



149 150

To a solution of **149** (15 g, 0.0158 mol) in anhydrous dichloromethane (265 mL) at −10 °C (bath temperature −15 °C), a solution of benzeneseleninic anhydride (7.4 g, 0.0143 mol) in anhydrous dichloromethane (265 mL) was added dropwise during 30 minutes, discarding any white solid present in the solution. The mixture was stirred for a further 10 minutes at the same temperature. The reaction was diluted with dichloromethane (200 mL) and a saturated aqueous sodium bicarbonate solution (500 mL) was added at −10 °C. The organic phase was separated, dried over sodium sulphate, filtered and concentrated to dryness at reduced pressure. The residue was purified by flash column chromatography eluting with mixtures of ethyl acetate and hexane in a gradient manner, from 1:2 to 100:0 to obtain **150** (14.20 g, 89%) as a yellow solid. The purified solid from chromatography is dissolved in dichloromethane (250 mL) and charcoal (4.95 g) was added. The suspension was then stirred at 23 °C for 1 hour. The mixture was filtered through a pad of celite and the celite was washed with dichloromethane (80 mL). The solvent was evaporated at reduced pressure to yield **150** (13.72 g, 86%) as a white solid. Rf = 0.37 Hex:EtOAc 1:2.

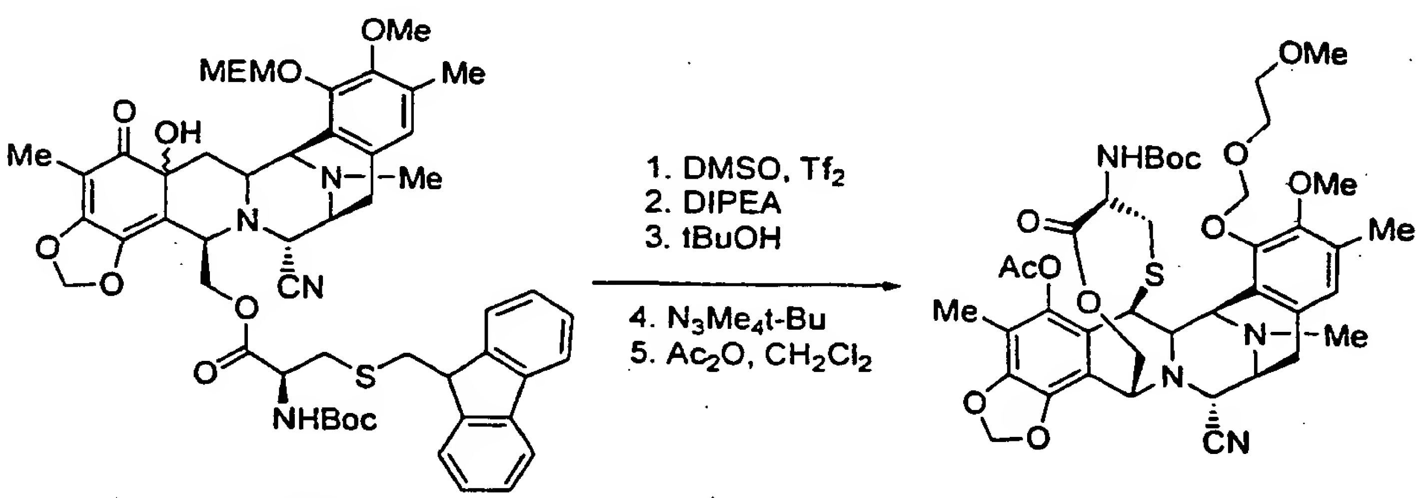
^1H NMR (300 MHz, $CDCl_3$) (mixture of isomers) δ 7.73 (t, J = 6.7 Hz, 4H), 7.63 (m, 2H), 7.54 (d, J = 7.6 Hz, 2H), 7.40-7.34 (m, 4H), 7.31-7.27 (m, 4H), 6.62 (s, 2H), 5.86 (s, 1H), 5.81 (s, 1H), 5.75 (s, 1H), 5.72 (s, 1H), 5.70 (s, 1H), 5.35 (d, J = 5.9 Hz, 1H), 5.30 (d, J = 8.4 Hz, 1H), 5.23 (d, J = 5.9 Hz, 1H), 5.22 (d, J = 5.9 Hz, 1H), 5.13 (d, J = 5.9 Hz, 1H), 4.97 (d, J = 8.8 Hz, 1H), 4.43 (m, 2H), 4.20-4.01 (m, 8H), 3.97-3.86 (m, 4H), 3.82 (s, 3H), 3.80-3.74 (m, 1H), 3.69 (s, 3H), 3.66-3.64 (m, 4H), 3.54 (m, 2H), 3.38 (s, 3H), 3.35 (s, 3H), 3.34-2.90 (m, 8H), 2.60-2.31 (m, 4H), 2.27 (s, 3H), 2.25 (s, 3H), 2.21 (s, 3H), 1.97 (s, 3H), 1.94-1.81 (m, 2H), 1.77 (s, 3H), 1.43 (s, 9H), 1.41 (s, 9H).

^{13}C NMR (75 MHz, $CDCl_3$) (mixture of isomers) δ 200.2, 198.3, 170.7, 170.5, 160.0, 155.2, 154.9, 148.5, 148.4, 145.5, 142.1, 140.9, 138.3,

130.9, 130.5, 130.0, 129.8, 127.5, 126.9, 125.0, 124.9, 124.7, 123.8, 122.5, 119.8, 117.2, 116.7, 111.5, 108.1, 104.6, 104.3, 101.3, 100.9, 98.0, 80.1, 72.1, 71.5, 70.5, 69.2, 69.0, 66.4, 63.5, 60.7, 60.1, 59.6, 58.9, 58.8, 58.0, 56.7, 56.4, 56.2, 55.9, 55.5, 55.0, 53.5, 46.7, 41.7, 41.3, 41.1, 36.9, 35.2, 35.1, 31.4, 28.1, 25.4, 25.3, 22.5, 15.7, 15.6, 14.0, 7.2.

ESI-MS m/z: Calcd. for $C_{52}H_{60}N_4O_{12}S$: 964.4. Found: 965.3 $(M+1)^+$, 987.3 $(M+23)^+$.

Example 76



150 **151**

The reaction flask was flamed twice, purged vacuum/Argon several times and kept under Argon atmosphere for the reaction. To a solution of DMSO (385.0 μL) in anhydrous CH_2Cl_2 (42 mL) was dropwise added triflic anhydride (366.5 μL, 2.16 mmol) at -78 °C. The reaction mixture was stirred at -78 °C for 20 minutes. Then, a solution of **150** (1 g, 1.03 mmol) in anhydrous CH_2Cl_2 (10 mL, for the main addition and 5 mL for washing) was added *via* canula (addition time: 5 min) at -78 °C. During the addition the temperature was kept at -78 °C in both flasks and the color changed from yellow to brown. The reaction mixture was stirred at -40 °C for 35 minutes. During this period of time the solution was turned from yellow to dark green. After this time, $^iPr_2NEt$ (1.51 mL, 9.55 mmol) was dropwise added and the reaction mixture was kept at 0 °C for 45 minutes, the color of the solution turned brown during this time.

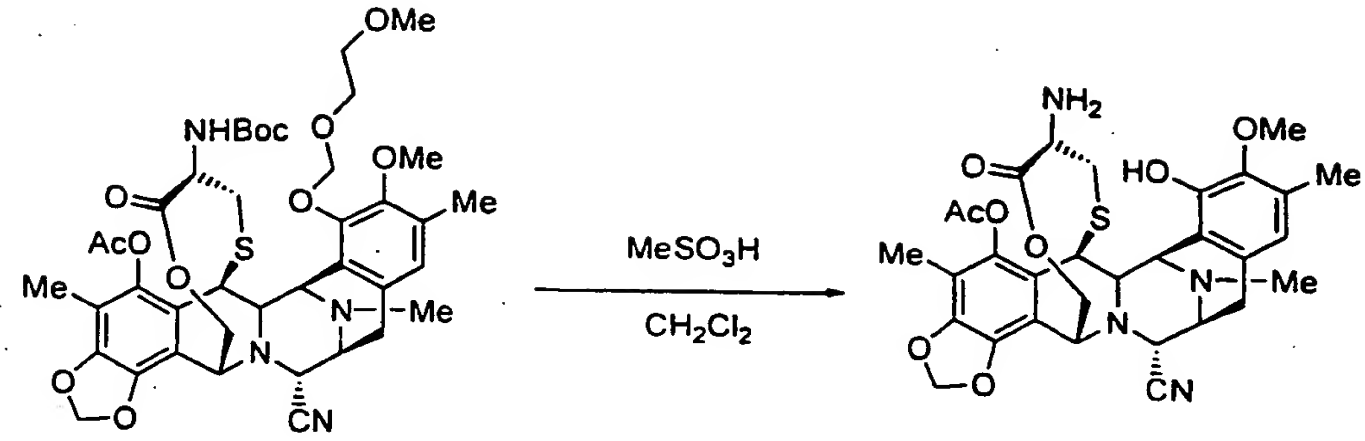
Then, ^{t}BuOH (409.5 ☐L, 4.33 mmol) and *tert*-butyl tetramethyl guanidine (1.31 mL, 7.61 mmol) were dropwise added and the reaction mixture was stirred at 23 °C for 40 minutes. After this time, acetic anhydride (1.03 mL, 10.89 mmol) was dropwise added and the reaction mixture was kept at 23 °C for 1 hour more. Then, the reaction mixture was diluted with CH_2Cl_2 (25 mL) and washed with aqueous saturated solution of NH_4Cl (50 mL), $NaHCO_3$ (50 mL), and NaCl (50 mL). The combined organic layers were dried over Na_2SO_4, filtered and concentrated. The residue was purified by flash column chromatography (inner diameter: 2.0 cm, height of silica: 9 cm; eluent: ethyl acetate/hexane in a gradient manner, from 20:80, 30:70 to 40:60) to afford **151** (832.6 mg, 99%) as a white solid. Rf = 0.48 Hex:EtOAc 3:2.

^{1}H NMR (300 MHz, $CDCl_3$) δ 6.78 (s, 1H), 6.09 (d, J = 1.2 Hz, 1H), 5.99 (d, J = 1.2 Hz, 1H), 5.32 (d, J = 5.8 Hz, 1H), 5.19 (d, J = 5.6 Hz, 1H), 5.01 (d, J = 11.7 Hz, 1H), 4.62 (d, J = 9.8 Hz, 1H), 4.50 (bs, 1H), 4.34 (d, J = 5.1 Hz, 1H), 4.28 (dd, J_1 = 2.4 Hz, J_2 = 6.8 Hz, 1H), 4.24 (s, 1H), 4.17 (m, 2H), 3.90 (m, 2H), 3.76 (s, 3H), 3.58 (t, J = 4.8 Hz, 2H), 3.42-3.37 (m, 2H), 3.37 (s, 3H), 2.91 (m, 2H), 2.36-2.08 (m, 2H), 2.30 (s, 3H), 2.28 (s, 3H), 2.21 (s, 3H), 2.04 (s, 3H), 1.44 (s, 9H).

^{13}C NMR (75 MHz, $CDCl_3$) δ 170.9, 168.9, 168.0, 155.4, 149.8, 148.6, 146.0, 141.1, 140.6, 131.6, 131.1, 130.6, 129.0, 125.1, 120.6, 118.3, 102.2, 98.4, 79.9, 71.9, 69.4, 61.6, 60.4, 59.8, 59.4, 59.2, 54.9, 54.7, 54.0, 41.6, 30.6, 29.1, 28.7, 23.9, 23.2, 20.6, 16.1, 14.2, 11.2, 9.8.

ESI-MS m/z: Calcd. for $C_{40}H_{50}N_4O_{12}S$: 810.91. Found $(M+1)^+$: 811.3.

Example 77



151 35

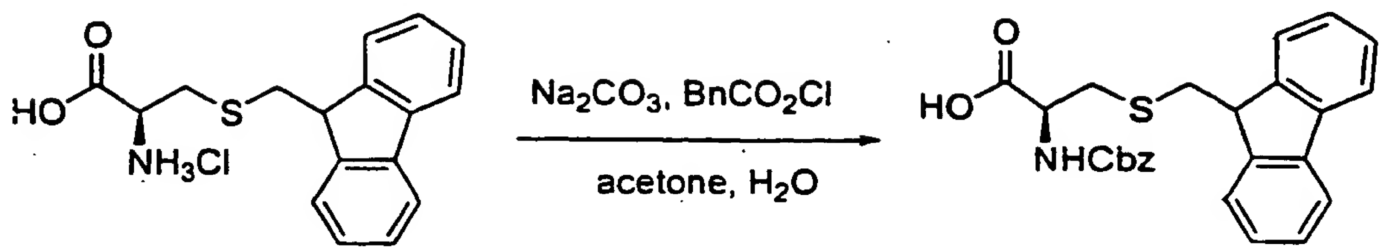
To a solution of **151** (2.9 g, 3.57 mmol) in CH_2Cl_2 (120 mL), $MeSO_3H$ (1.4 mL, 21.46 mmol) was added at 23°C. After stirring the reaction for 30 minutes at 23°C, a saturated aqueous sodium bicarbonate solution (200 mL) was added at 0 °C. The organic phase was separated, dried over sodium sulphate, filtered and concentrated to dryness at reduced pressure. The residue was purified by flash column chromatography, eluting with mixtures of ethyl acetate and hexane in a gradient manner, from 0:1 to 1:0 to obtain **35** (1.43 g, 64%) as a pale yellow solid. Experimental data of **35** was previously decribed in PCT/GB00/01852.

36, **ET-770** and **ET-743** were prepared following the same procedures than those previously described in PCT/GB00/01852.

Route 3

The first step of this Route (transformation of **21** into **146**) was described above in Example 71.

Example 78



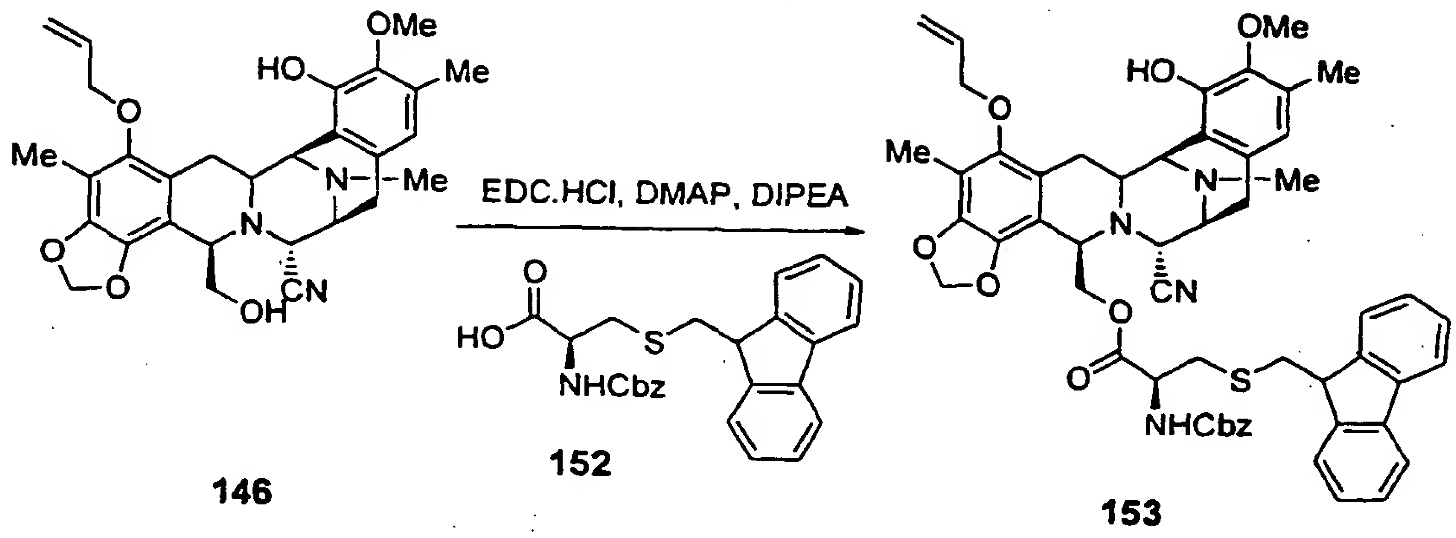
HO.Cys(Fm)-H.HCl

152

To a solution of the commercial available HO.Cys(Fm)-H.HCl (Bachem) (40 g, 0.119 mol) in acetone (500 mL) and water (500 mL), 1M Na_2CO_3 solution (238 mL) and $BnCO_2Cl$ (18.7 mL, 0.131 mol) were added at 0°C. After stirring the reaction at 60°C for 30 minutes, the mixture was quenched with 1N HCl (pH 0 1) and extracted with eter (3 x 400 mL). The organic phase was separated, dried over magnesium sulphate filtered and concentrated to dryness at reduced pressure. The crude solid was disolved in a mixture of $EtOAc/CH_2Cl_2$ 1:1, precipitated with hexane and kept at 4°C overnight. Then, the suspension was filtered off, the solid washed with hexane (200 mL) and the filtrate was dried *in vacuo* to afford **152** (50.16 g, 97%) as a white solid.

^{1}H NMR (300 MHz, $CDCl_3$) δ 10.66 (bs, 1H), 7.74 (d, J = 7.5 Hz, 2H), 7.69-7.64 (m, 2H), 7.62-7.29 (m, 9H), 5.67 (d, J = 7.5 Hz, 1H), 5.14 (bs, 2H), 4.70-4.64 (m, 1H), 4.09-4.05 (m, 1H), 3.12-3.09 (m, 2H).
^{13}C NMR (75 MHz, $CDCl_3$) δ 175.2, 155.9, 145.5, 141.0, 135.8, 128.5, 128.2, 128.1, 127.5, 127.0, 124.7, 119.8, 84.8, 67.3, 46.8, 37.0.
ESI-MS m/z: Calcd. for $C_{25}H_{23}NO_4S$: 433.52. Found $(M+1)^+$: 434.4.

Example 79

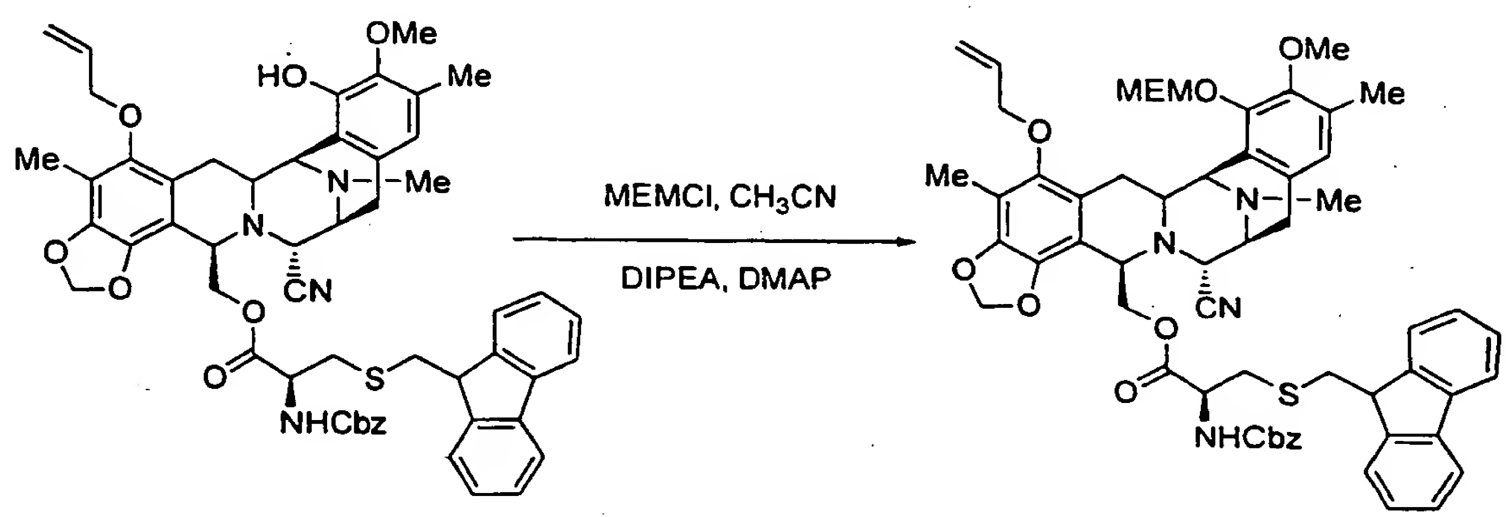


To a stirred solution of **146** (10 g, 19.2 mmol) and **152** (12.5 g, 28.8 mmol) in dichloromethane (800 mL) under argon, dimethylaminopyridine (705 mg, 5.77 mmol), 1-[3-(Dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride (9.2 g, 48.1 mmol) and diisopropylethy amine (7.4 mL, 42.3 mmol) were added dropwise during 1 h at 0 °C. The mixture was then stirred at 23 °C for 1.5 more hour. The reaction was quenched by addition of a saturated aqueous sodium bicarbonate solution (600 mL). The organic phase was separated and washed again with a saturated aqueous amonium chloride solution (500 mL) and a saturated sodium chloride solution (500 mL). The organic extract were dried over sodium sulphate, filtered and evaporated to dryness under reduced pressure. The crude product was purified by flash column chromatography (RP18, CH_3CN:H_2O 4:1) to yield **153** (13.89 g, 77%) as a pale yellow solid.

^{1}H NMR (300 MHz, $CDCl_3$) δ 7.74-7.72 (m, 2H), 7.61-7.53 (m, 2H), 7.37-7.24 (m, 9H), 6.39 (s, 1H), 6.09-5.96 (m, 1H), 5.90 (s, 1H), 5.84 (s, 1H), 5.78 (s, 1H), 5.34 (dd, J_1 = 1.5 Hz, J_2 = 17.4 Hz, 1H), 5.32 (bs, 1H), 5.24 (dd, J_1 = 1.5 Hz, J_2 = 10.2 Hz, 1H), 5.17-5.07 (m, 2H), 4.40 (dd, J_1 = 3.6 Hz, J_2 = 10.8 Hz, 1H), 4.30 (m, 1H), 4.18-4.01 (m, 6H), 3.92 (brt, J = 6.3 Hz, 1H), 3.71 (s, 3H), 3.30-3.19 (m, 3H), 2.99-2.85 (m, 3H), 2.65 (dd, J_1 = 4.5 Hz, J_2 = 14.4 Hz, 1H), 2.55 (d, J = 18.3 Hz, 1H), 2.26 (s, 3H), 2.21 (s, 3H), 2.06 (s, 3H), 1.86 (dd, J_1 = 11.7 Hz, J_2 = 15.9 Hz, 1H).

^{13}C NMR (75 MHz, $CDCl_3$) δ 170.2, 155.6, 148.6, 146.8, 145.7, 145.6, 144.3, 142.6, 140.7, 139.0, 133.7, 131.1, 128.8, 128.4, 128.1, 128.0, 127.4, 126.9, 124.7, 124.6, 121.0, 120.5, 119.7, 117.8, 117.3, 116.8, 112.5, 112.0, 101.0, 74.1, 67.0, 64.7, 60.7, 59.9, 57.0, 56.6, 56.3, 55.2, 53.1, 46.5, 41.4, 36.4, 34.8, 26.2, 24.8, 15.6, 9.2.
ESI-MS m/z: Calcd. for $C_{54}H_{54}N_4O_9S$: 934.36. Found $(M+1)^+$: 935.4.

Example 80



153 154

To a solution of **153** (13.89 g, 14.85 mmol) in CH_3CN (74.3 mL), MEMCl (25.4 mL, 223 mmol), DIPEA (52 mL, 297 mmol) and DMAP (0.181 g, 0.15 mmol) were added at 0°C. The mixture was stirred for 5 h at 23°C. The reaction was diluted with CH_2Cl_2 (400 mL) and extracted with 0.1N HCl (300 mL) and 3N HCl (pH = 3). The aqueous phase was extracted again with CH_2Cl_2 (2 x 50 mL). The combined organic phases were dried over Na_2SO_4, filtered and concentrated *in vacuo* to give a residue which was purified by flash column chromatography (SiO_2, CH_2Cl_2:EtOAc 10:1, 5:1) to give **154** (13.47 g, 88%) as a white solid. Rf= 0.27 CH_2Cl_2:AcOEt 6:1.

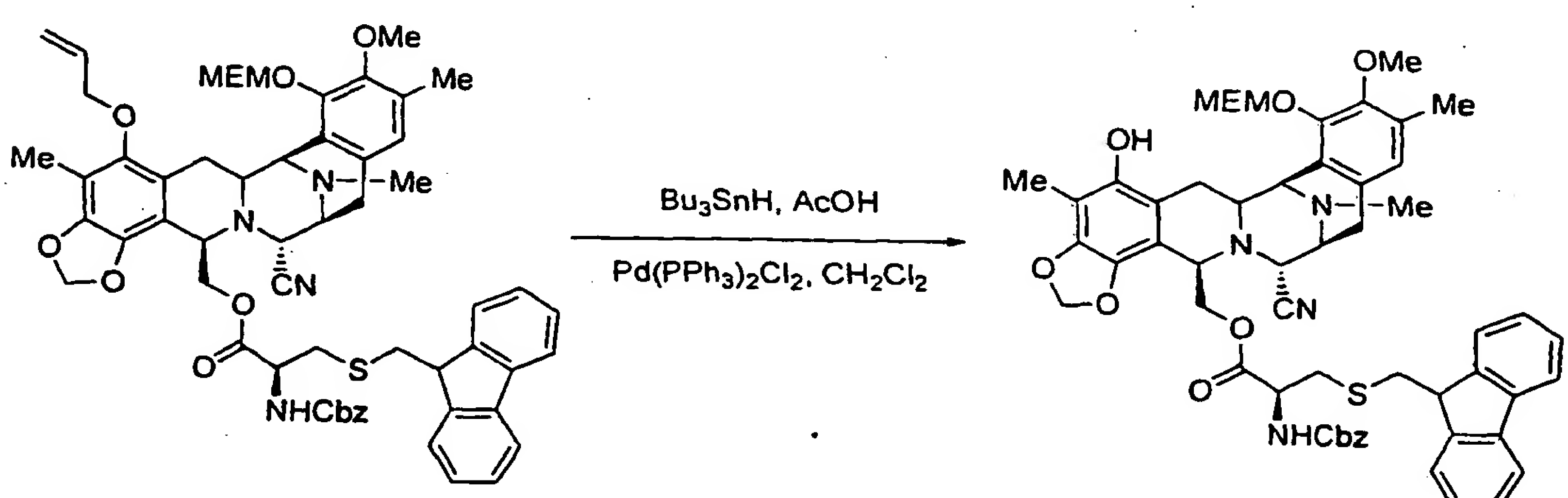
^1H NMR (300 MHz, $CDCl_3$) δ 7.73-7.70 (m, 2H), 7.58-7.50 (m, 2H), 7.38-7.22 (m, 9H), 6.59 (s, 1H), 6.08-5.98 (m, 1H), 5.89 (s, 1H), 5.77 (s, 1H), 5.35 (d, J = 17.1 Hz, 1H), 5.31-5.28 (m, 1H), 5.23 (d, J = 6.9 Hz, 1H),

5.13 (d, J = 6.9 Hz, 1H), 5.12-5.05 (m, 2H), 4.37-4.29 (m, 2H), 4.15-3.77 (m, 9H), 3.68 (s, 3H), 3.58-3.55 (m, 2H), 3.37 (s, 3H), 3.30-3.27 (m, 1H), 3.21-3.16 (m, 2H), 2.96-2.84 (m, 4H), 2.64-2.58 (m, 1H), 2.55 (d, J = 18 Hz, 1H), 2.27 (s, 3H), 2.16 (s, 3H), 2.02 (s, 3H), 1.75 (dd, J_1 = 12.3 Hz, J_2 = 16.2 Hz, 1H).

^{13}C NMR (75 MHz, $CDCl_3$) δ 171.9, 170.2, 155.5, 148.7, 148.6, 148.3, 145.8, 145.7, 144.5, 142.1, 140.9, 139.1, 136.1, 133.8, 130.8, 130.5, 128.5, 128.3, 128.1, 127.6, 127.0, 124.9, 124.7, 123.9, 122.2, 120.9, 119.8, 117.8, 117.3, 112.6, 112.0, 101.1, 98.2, 74.0, 71.7, 69.3, 67.1, 65.1, 60.1, 59.8, 59.0, 56.9, 56.8, 56.7, 55.3, 53.3, 46.7, 41.4, 36.5, 35.0, 31.6, 29.7, 26.4, 25.0, 22.6, 15.7, 14.1, 9.2.

ESI-MS m/z: Calcd. for $C_{58}H_{62}N_4O_{11}S$: 1023.2. Found $(M+23)^+$: 1046.3.

Example 81



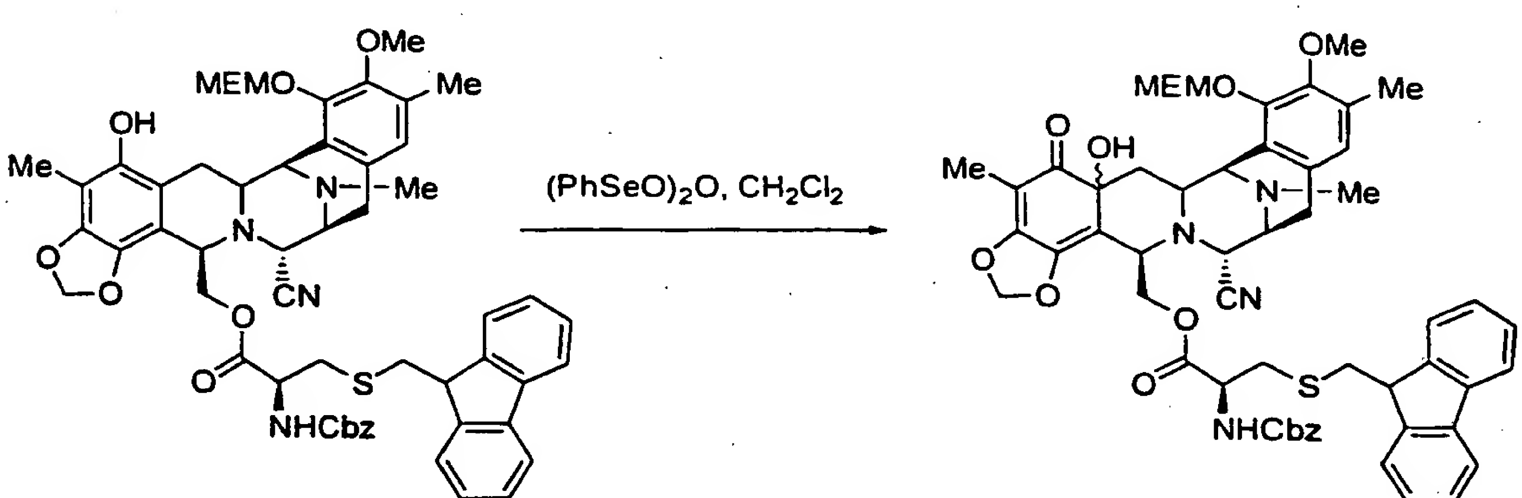
154 155

To a stirred solution of **154** (20.84 g. 0.02 mol) in dichloromethane (530 mL), dichlorobis(triphenylphosphine) palladium (II) (1.14 g, 1.63 mmol) and acetic acid (11.64 mL, 0.2 mol) were added under argon at 23 °C. Then, tributyltin hydride (27.44 mL, 0.1 mol) was added in a dropwise manner. The mixture was stirred at 23 °C for 15 minutes. The reaction was then filtered through a silica gel column compacted with hexane. **155** (18.78 g, 94%) was obtained as a pale yellow solid by subsequent elution with mixtures of ethyl acetate and hexane in a gradient manner, from 1:4, 1:1, 3:2 to 7:3.

^{1}H NMR (300 MHz, $CDCl_3$) δ 7.71 (d, J = 7.2 Hz, 2H), 7.59 (d, J = 7.5 Hz, 1H), 7.53 (d, J = 7.5 Hz, 1H), 7.41-7.23 (m, 9H), 6.60 (s, 1H), 5.87 (bs, 2H), 5.74 (s, 1H), 5.40 (d, J = 6.3 Hz, 1H), 5.33 (d, J = 5.8 Hz, 1H), 5.18 (d, J = 9 Hz, 1H), 5.09 (d, J = 12 Hz, 1H), 4.97 (d, J = 12 Hz, 1H), 4.56 (dd, J_1 = 3 Hz, J_2 = 11.1 Hz, 1H) 4.19 (d, J = 2.1 Hz, 1H), 4.16-3.87 (m, 9H), 3.66 (s, 3H), 3.38 (s, 3H), 3.32-3.20 (m, 3H), 2.96-2.87 (m, 3H), 2.62-2.54 (m, 2H), 2.28 (s, 3H), 2.19 (s, 3H), 1.97 (s, 3H), 1.82 (dd, J_1 = 13.2 Hz, J_2 = 15.6 Hz, 1H).

^{13}C NMR (75 MHz, $CDCl_3$) δ 170.0, 155.4, 149.0, 147.5, 145.7, 145.6, 144.4, 140.8, 135.9, 130.9, 128.4, 128.1, 128.0, 127.4, 126.9, 124.7, 124.6, 122.7, 119.7, 117.7, 112.4, 111.4, 100.6, 98.7, 71.5, 69.4, 67.0, 64.9, 63.9, 59.7, 59.6, 58.8, 57.0, 56.5, 56.4, 55.1, 54.9, 53.1, 52.5, 46.5, 41.4, 36.8, 34.9, 25.8, 24.7, 15.7, 8.7.

Example 82



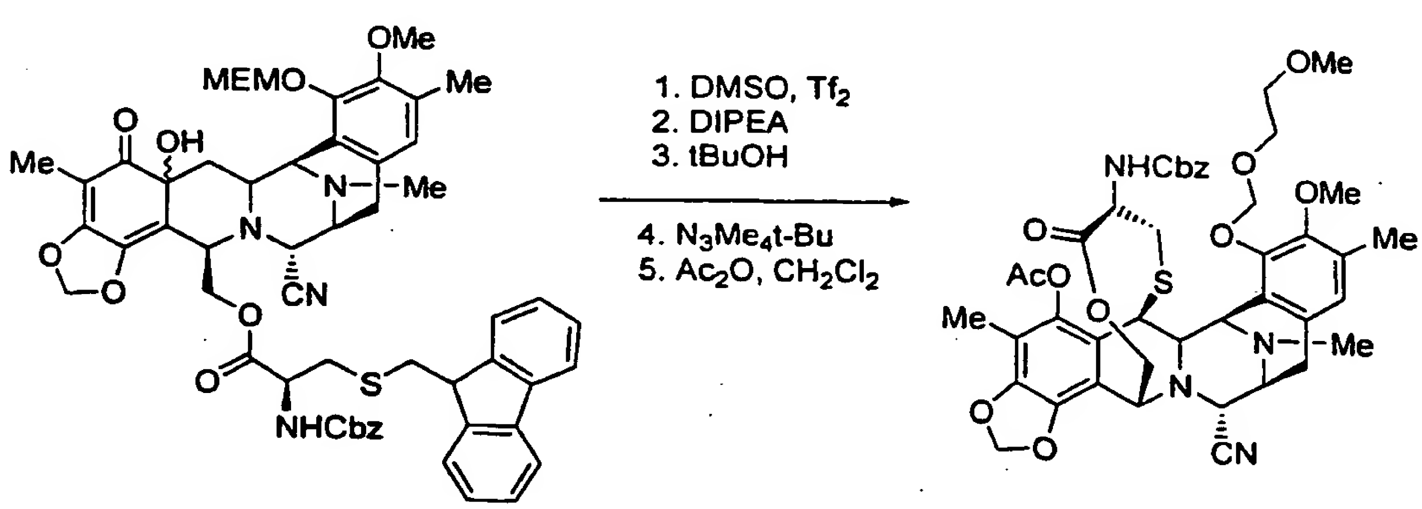
155 156

To a solution of **155** (18.5 g, 18.82 mmol) in anhydrous dichloromethane (530 mL) at −10 °C (bath temperature −15 °C), a solution of benzeneseleninic anhydride (9.68 g, 18.82 mmol) in anhydrous dichloromethane (290 mL) was added dropwise, discarding any white solid present in the solution. The mixture was stirred for 10 minutes at the same temperature. The reaction was then quenched with a saturated aqueous sodium bicarbonate solution (600 mL). The

organic phase was separated, and the aqueous phase was extracted with CH_2Cl_2 (2 x 300 mL). The combined organic extracts were dried over sodium sulphate, fitltered, and concentrated to dryness under reduced pressure. The residue was purified by column chromatography, eluting with mixtures of ethyl acetate and hexane in a gradient manner, from 1:1, 3:2, 7:3 to 4:1 to obtain **156** (17.62 g, 88%) as a pale yellow solid.

^1H NMR (300 MHz, $CDCl_3$) (mixture of isomers) δ 7.73 (d, J = 7.5 Hz, 2H), 7.63 (d, J = 7.5 Hz, 2H), 7.40-7.29 (m, 9H), 6.59 (s, 1H), 6.52 (s, 1H), 5.68 (s, 1H), 5.66 (s, 1H), 5.58 (s, 1H), 5.56 (s, 1H), 5.23 (d, J = 6 Hz, 1H), 5.15-5.05 (m, 4H), 4.76-4.68 (m, 1H), 4.64-4.55 (m, 1H), 4.40-4.37 (m, 1H), 4.15-3.68 (m, 8H), 3.60 (s, 3H), 3.57 (s, 3H), 3.39 (s, 3H), 3.36 (s, 3H), 3.25-2.78 (m, 7H), 2.38-2.24 (m, 2H), 2.20 (s, 3H), 2.18 (s, 3H), 2.15 (s, 3H), 2.09 (m, 1H), 2.04 (s, 3H), 1.77 (s, 3H), 1.58 (s, 3H). ESI-MS m/z: Calcd. for $C_{55}H_{58}N_4O_{12}S$: 999.13. Found $(M+1)^+$: 1000.0.

Example 83



156 **157**

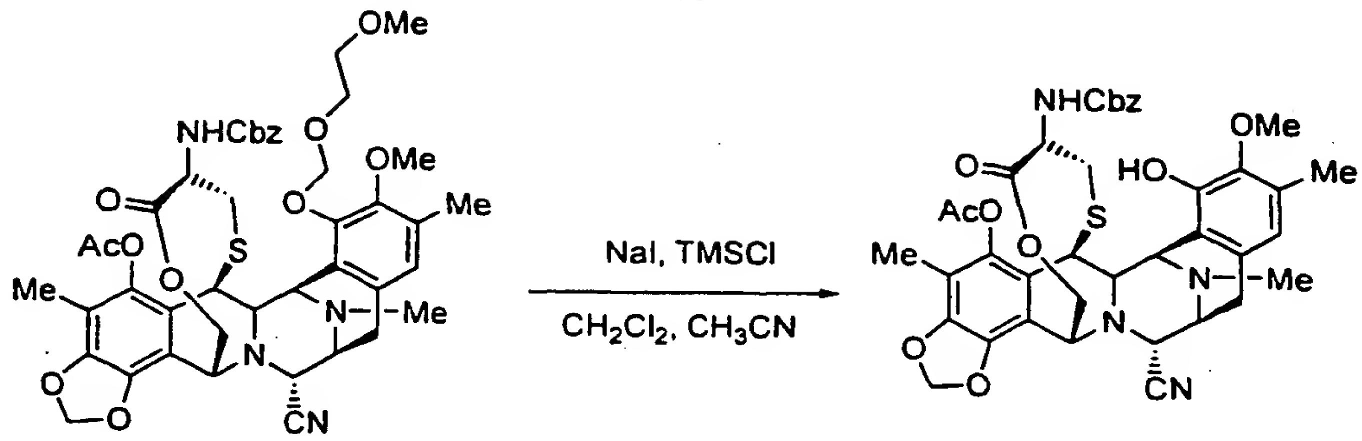
The reaction flask was flamed twice, purged vacuum/Argon several times and kept under Argon atmosphere for the reaction. To a solution of DMSO (178 μL) in anhydrous CH_2Cl_2 (20 mL) was dropwise added triflic anhydride (169 μL, 1 mmol) at -78 °C. The reaction mixture was

stirred at -78 °C for 20 minutes. Then, a solution of **156** (0.5 g, 0.5 mmol) in anhydrous CH_2Cl_2 (4 mL, for the main addition and 1.5 mL for washing) was added *via* canula (addition time: 5 min) at -78 °C. During the addition the temperature was kept at -78 °C in both flasks and the color changed from yellow to brown. The reaction mixture was stirred at -40 °C for 35 minutes. During this period of time the solution was turned from yellow to dark green. After this time, $^iPr_2NEt$ (0.7 mL, 4.42 mmol) was dropwise added and the reaction mixture was kept at 0 °C for 45 minutes, the color of the solution turned brown during this time. Then ^tBuOH (189 µL, 2 mmol) and *tert*-butyl tetramethyl guanidine (0.6 mL, 3.49 mmol) were dropwise added and the reaction mixture was stirred at 23 °C for 40 minutes. After this time, acetic anhydride (0.47 mL, 4.97 mmol) was dropwise added and the reaction mixture was kept at 23 °C for 1 hour more. Then, the reaction mixture was diluted with CH_2Cl_2 (15 mL) and washed with aqueous saturated solution of NH_4Cl (25 mL), $NaHCO_3$ (25 mL), and NaCl (25 mL). The combined organic layers were dried over Na_2SO_4, filtered and concentrated. The residue was purified by flash column chromatography (inner diameter: 2.0 cm, height of silica: 9 cm; eluent: ethyl acetate/hexane in a gradient manner, from 1:4, 1:3, 1:2 to 1:1) to afford **157** (128 mg, 30%) as a light yellow solid. Rf = 0.37 Hex:EtOAc 3:2.

^1H NMR (300 MHz, $CDCl_3$) δ 7.37 (bs, 5H), 6.66 (s, 1H), 6.09 (s, 1H), 5.99 (s, 1H), 5.30 (d, J = 5.4 Hz, 1H), 5.17 (d, J = 6 Hz, 1H), 5.06 (d, J = 7.8 Hz, 1H), 5.00 (s, 1H), 4.83 (d, J = 9.3 Hz, 1H), 4.50 (s, 1H), 4.34-4.17 (m, 7H), 3.90-3.87 (m, 2H), 3.66 (s, 3H), 3.65-3.56 (m, 2H), 3.37 (s, 3H), 2.89-2.90 (m, 2H), 2.28 (s, 3H), 2.18 (s, 3H), 2.15-2.04 (m, 2H), 2.03 (s, 3H), 1.99 (s, 3H).

ESI-MS m/z: Calcd. for $C_{43}H_{48}N_4O_{12}S$: 844.93. Found $(M+1)^+$: 845.8.

Example 84



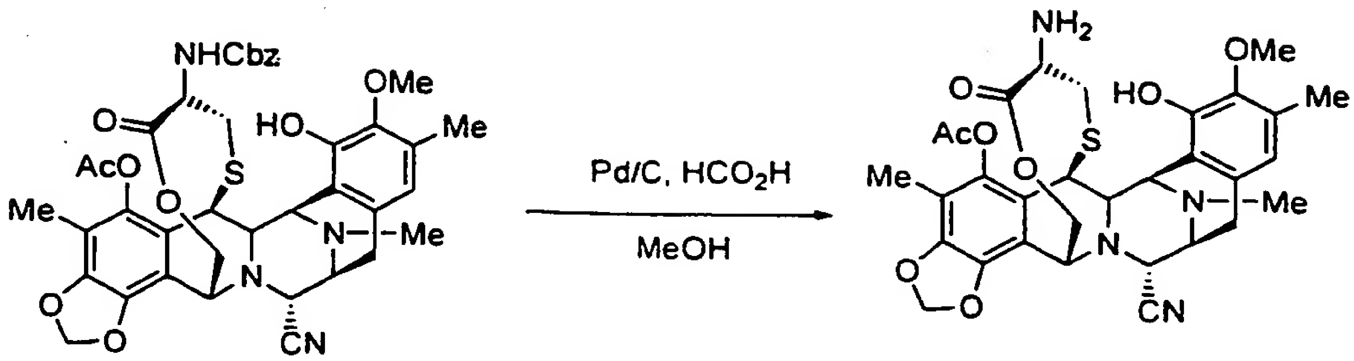
157

158

To a solution of **157** (100 mg, 0.118 mmol) in CH_2Cl_2 (2 mL) and CH_3CN (2 mL), NaI (71 mg, 0.472 mmol) and TMSCl (60 □L, 0.472 mmol) were added at 0°C. After stirring the reaction at 23°C for 50 minutes, the mixture was quenched with water (30 mL) and extracted with CH_2Cl_2 (2 x 20 mL). The combined organic phases were washed successively with a saturated solution of NaCl (20 mL) and a saturated solution of sodium ditionite (20 mL), dried over Na_2SO_4, filtered and concentrated *in vacuo*. The residue was purified by flash column chromatography (eluent: ethyl acetate/hexane gradient from 1:4, 1:2 to 1:1) to afford **158** (62 mg, 70%) as white solid. Rf = 0.21 Hex:EtOAc 1:1.

^1H NMR (300 MHz, $CDCl_3$) δ 7.36 (bs, 5H), 6.44 (s, 1H), 6.07 (d, J = 1.2 Hz, 1H), 5.97 (d, J = 1.2 Hz, 1H), 5.81 (bs, 1H), 5.10-5.00 (m, 3H), 4.82 (d, J = 9.3 Hz, 1H), 4.49 (bs, 1H), 4.35-4.30 (m, 1H), 4.21-4.17 (m, 2H), 4.16-4.14 (m, 2H), 3.65 (s, 3H), 3.41-3.36 (m, 2H), 2.88-2.85 (m, 2H), 2.28 (s, 3H), 2.24-2.03 (m, 2H), 2.17 (s, 3H), 2.02 (s, 3H), 2.00 (s, 3H).
^{13}C NMR (75 MHz, $CDCl_3$) δ 170.5, 168.8, 155.9, 148.3, 146.0, 143.1, 141.2, 140.6, 136.6, 130.6, 130.0, 128.8, 128.7, 128.5, 121.0, 120.3, 118.3, 118.2, 113.7, 113.6, 102.2, 67.2, 61.5, 60.8, 60.3, 59.6, 59.5, 54.8, 54.7, 54.1, 41.9, 41.6, 32.9, 23.9, 20.8, 15.5, 9.8.
ESI-MS m/z: Calcd. for $C_{39}H_{40}N_4O_{10}S$: 756.82. Found $(M+1)^+$: 757.3.

Example 85



158

35

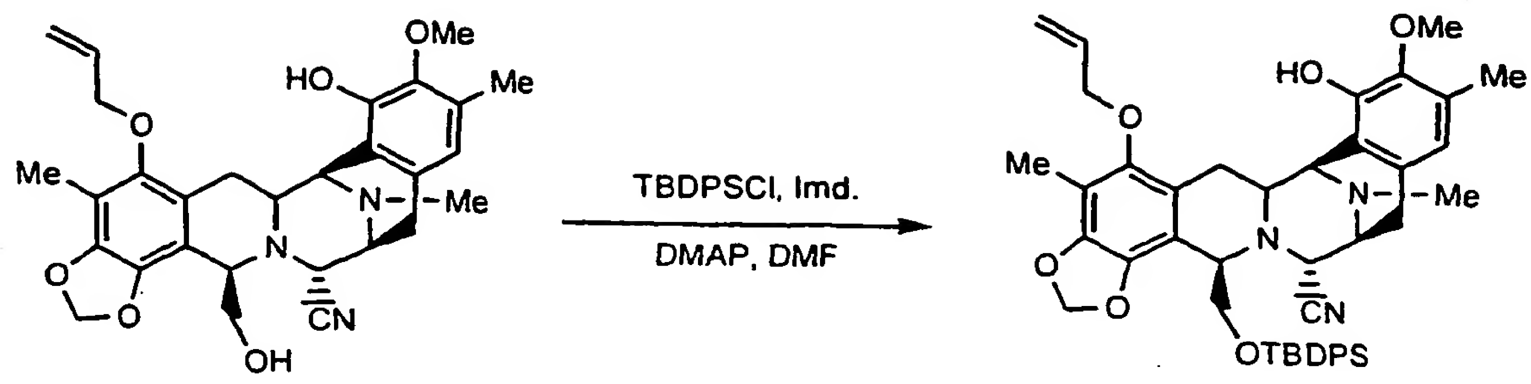
To a solution of **158** (100 mg, 0.132 mmol) in MeOH (6.8 mL), HCO_2H (360 µL) and 10% Pd/C (140 mg, 0.132 mmol) were added at 23 °C and the mixture was stirred for 15 minutes. Then, toluene (7 mL) was added to the reaction and the solvent was evaporated under reduced pressure. The azeotropic destillation with toluene was repeated 3 times. The residue was then diluted with dichloromethane (15 mL) and a saturated aqueous solution of sodium bicarbonate (15 mL) was added. The aqueous phase was separated and extracted with dichloromethane (2 x 10 mL). The combined organic extracts were dried over sodium sulphate, filtered and evaporated to dryness under reduced pressure. The residue was then purified by flash column chromatography on amino-silicagel and eluting with mixtures of ethyl acetate and hexane in a gradient manner, from 1:2, 1:1 to 2:1 to give **35** (57 mg, 70%) as a yellow solid. Experimental data of **35** were previously described in PCT/GB00/01852.

36, **ET-770** and **ET-743** were prepared following the same procedures than those previously described in PCT/GB00/01852.

Route 4

The first step of this Route (transformation of **21** into **146**) was described above in Example 71.

Example 86



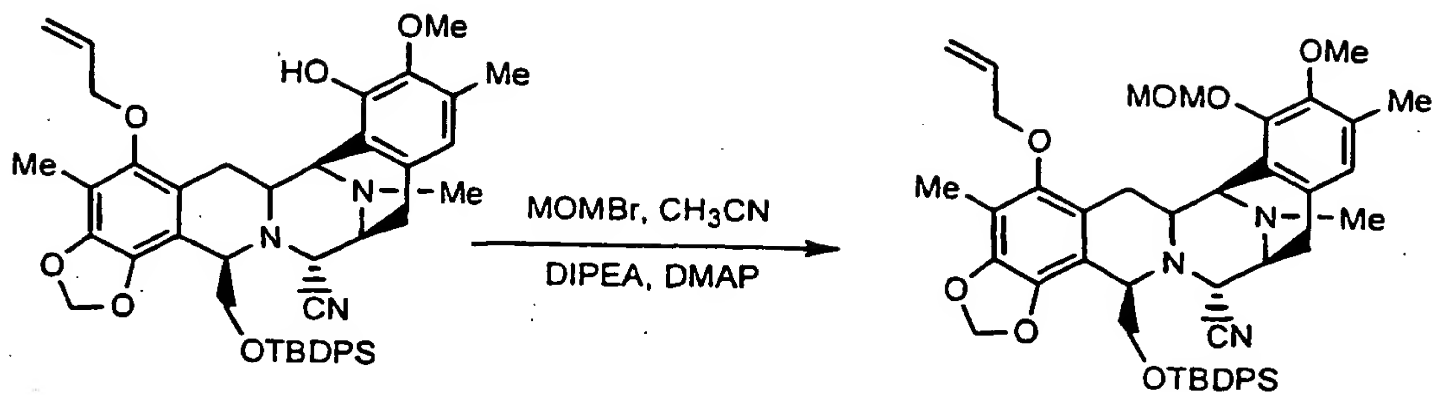
To a solution of **146** (18 mg, 0.032 mmol), cat. DMAP and imidazole (5 mg, 0.08 mmol) in DMF (0.05 mL) at 0°C, tert-buthyldiphenylsilyl chloride (12.5 μL, 0.048 mmol) was added and the reaction was stirred for 4 hours at 23°C.Then, water (30 mL) was added at 0°C and the mixture was extracted with Hex:EtOAc 1:10 (2 x 40 mL). The combined organic phases were dried over sodium sulphate, filtered, and the solvent was removed under reduced pressure. The residue was puified by flash column chromatography (SiO_2, Hex:EtOAc 3:1) to afford **159** (27 mg, 88%) as a white solid. Rf = 0.29 Hex:EtOAc 3:1.

^1H NMR (300 MHz, $CDCl_3$) δ 7.72-7.41 (m, 2H), 7.40-7.20 (m, 8H), 6.46 (s, 1H), 6.16-6.00 (m, 1H), 5.77 (d, J = 1.5 Hz, 1H), 5.71 (s, 1H), 5.63 (d, J = 1.5 Hz, 1H), 5.24 (dd, J_1 = 1.2 Hz, J_2 = 17.1 Hz, 1H), 5.23 (dd, J_1 = 1.2 Hz, J_2 = 10.2 Hz, 1H), 4.18 (d, J = 2.4 Hz, 1H), 4.13-4.00 (m, 4H), 3.77 (s, 3H), 3.63 (dd, J_1 = 2.4 Hz, J_2 = 7.5 Hz, 1H), 3.39-3.19 (m, 4H), 2.99 (dd, J_1 = 8.1 Hz, J_2 = 18.0 Hz, 1H), 2.68 (d, J = 17.7 Hz, 1H), 2.30 (s, 3H), 2.28 (s, 3H), 2.08 (s, 3H), 1.99 (dd, J_1 = 12.6 Hz, J_2 = 16.3 Hz, 1H), 0.89 (s, 9H).

^{13}C NMR (75 MHz, $CDCl_3$) δ 148.3, 146.6, 144.0, 142.5, 139.0, 135.7, 135.4, 133.9, 133.6, 132.2, 131.2, 129.5, 129.4, 128.3, 127.5, 127.4, 121.8, 120.9, 118.7, 117.3, 117.2, 112.9, 111.7, 100.8, 74.2, 68.0, 61.6, 60.6, 60.3, 59.0, 57.4, 56.7, 55.4, 41.7, 29.6, 26.6, 26.5, 25.5, 18.9, 15.8, 9.3.

ESI-MS m/z: Calcd. for $C_{45}H_{51}N_3O_6Si$: 757.9. Found $(M+1)^+$: 758.4.

Example 87

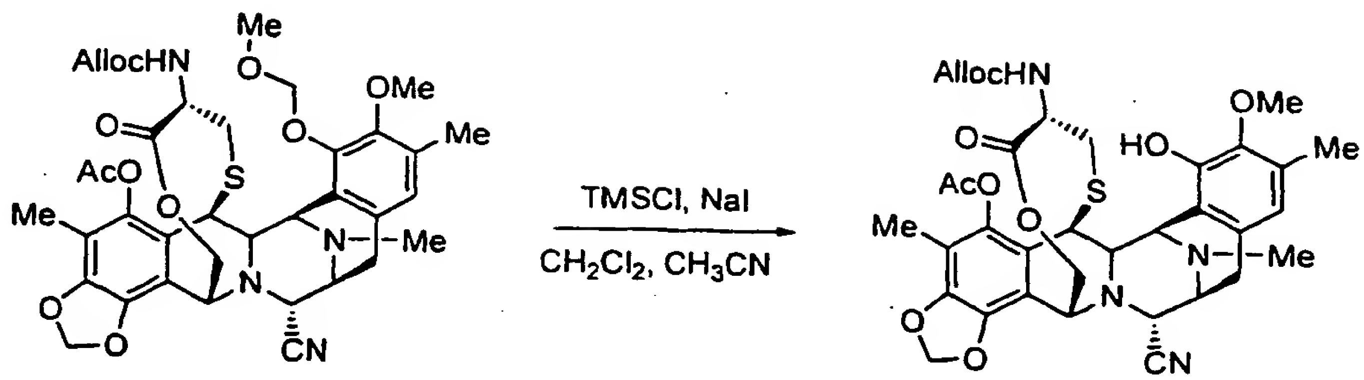


159 **26**

To a solution of **159** (2.4 g, 3.17 mmol) in CH_3CN (16 mL), MOMBr (2.6 mL, 31.75 mmol), DIPEA (8.3 mL, 47.6 mmol) and DMAP (16 mg, 0.127 mmol) were added at 0°C. The mixture was stirred for 6 h at 23°C. The reaction was diluted with CH_2Cl_2 (50 mL) and extracted with 0.1N HCl (50 mL). The aqueous phase was extracted again with CH_2Cl_2 (50 mL). The combined organic phases were dried over Na_2SO_4, filtered and concentrated *in vacuo* to give a residue which was purified by flash column chromatography (SiO_2, CH_2Cl_2:EtOAc 15:1, 5:1) to give **26** (1.78 g, 70%) as a white solid. Experimental data of **26** were described previously in PCT/GB00/01852.

Experimental procedures for **Int. 11**, **160**, **161**, **162**, and **163** were previously described in U. S. Patent No 5,721,362.

Example 88



163 164

To a solution of **163** (15.8 g, 0.02 mol) in anhydrous CH_2Cl_2 (250 mL) and acetonitrile (300 mL), NaI (31.5 g, 0.21 mol) and ClTMS (freshly distilled over CaH_2, 26.7 mL, 0.21 mol) were added under argon atmosphere at 23°C. The reaction mixture was stirred for 40 minutes. Then the reaction was partitioned between CH_2Cl_2 (200 mL) and water (300 mL). The organic layer was washed with a saturated aqueous solution of NaCl (2 x 300 mL). The organic phase was dried over Na_2SO_4, filtered and the solvent was eliminated under reduced pressure. The crude was purified by flash column chromatography using ethyl acetate/hexane 2:3 as eluent to afford **164** (10.74 g, 76%) as a pale yellow solid. Rf = 0.25 Hex:EtOAc 3:2.

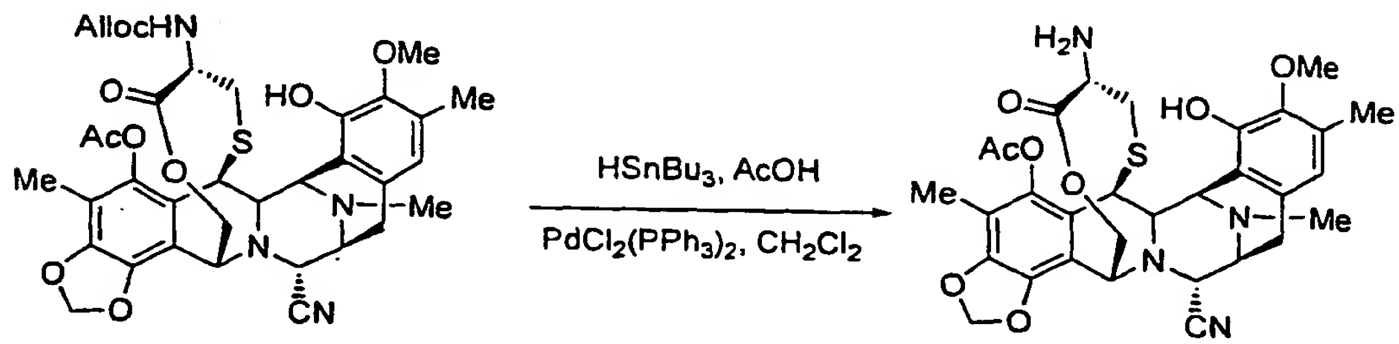
^1H NMR (300 MHz, $CDCl_3$) δ 6.57 (s,1H), 6.08 (d, J = 1.5 Hz, 1H), 5.98 (d, J = 1.5 Hz, 1H), 5.96-5.85 (m, 1H), 5.76 (bs, 1H), 5.30 (dd, J_1 = 1.5, J_2 = 17.3 Hz, 1H), 5.23 (dd, J_1 = 1.5, J_2 = 10.2 Hz, 1H), 5.00 (d, J = 12.1 Hz, 1H), 4.81 (d, J = 9.8 Hz, 1H), 4.58-4.45 (m, 3H), 4.34-4.28 (m, 1H), 4.23 (m, 2H), 4.17-4.00 (m, 2H), 3.76 (s, 3H), 3.40-3.38 (m, 2H), 2.91-2.85 (m, 2H), 2.30 (s, 3H), 2.29 (s, 3H), 2.24-2.23 (m, 2H), 2.19 (s, 3H), 2.02 (s, 3H).

^{13}C NMR (75 MHz, $CDCl_3$) δ 170.1, 168.4, 155.2, 148.0, 145.5, 142.8, 140.7, 140.1, 132.7, 130.2, 129.6, 120.7, 119.9, 117.8, 113.3, 101.9,

65.6, 61.0, 60.4, 59.9, 59.2, 59.0, 54.3, 53.6, 41.5, 41.2, 32.6, 29.5, 23.5, 20.4, 15.6, 9.4.

ESI-MS m/z: Calcd. for $C_{35}H_{38}N_4O_{10}S$: 706.76. Found $(M+1)^+$: 707.2.

Example 89



164 **35**

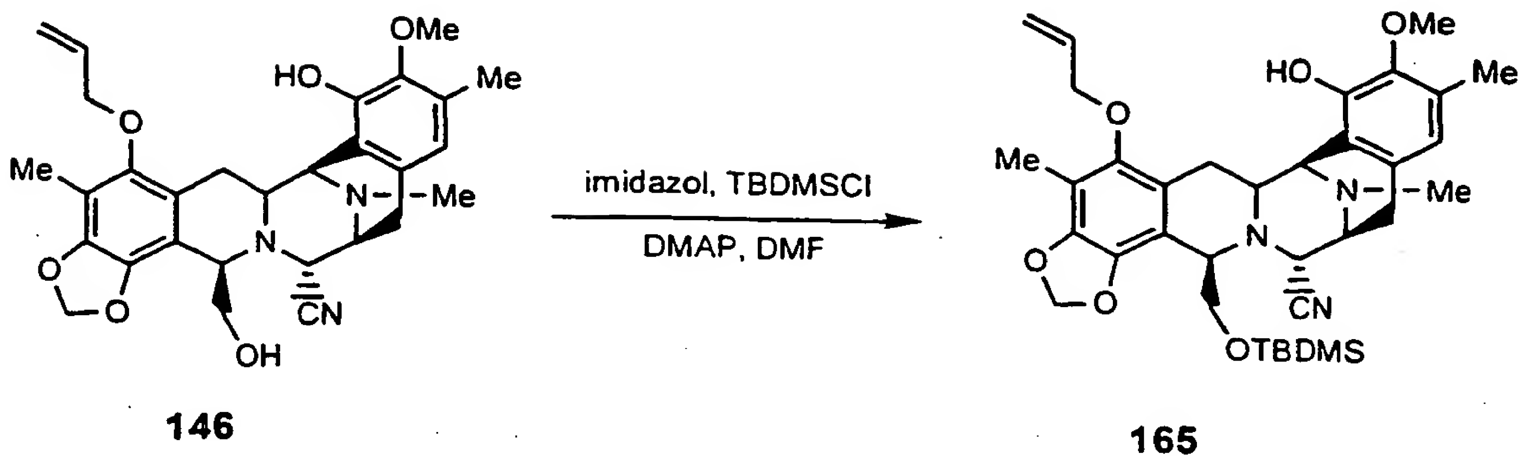
To a stirred solution of **164** (2 g. 2.85 mmol) in dichloromethane (142 mL), dichlorobis(triphenylphosphine) palladium (II) (0.2 g, 0.28 mmol) and acetic acid (0.65 mL, 11.4 mmol) were added under argon at 23 °C. Then, tributyltin hydride (4.51 mL, 17.02 mmol) was added in a dropwise manner during 25 minutes. After addition of $HsnBu_3$, the mixture was stirred at 23 °C for 20 minutes more. The reaction was filtered through a silical gel column compacted with hexane. **35** (1.38 g, 78%) was obtained by subsequent elution with mixtures of ethyl acetate and hexane in a gradient manner, from 1:2 to 15:1. Experimental data of **35** were previously described in PCT/GB00/01852.

36, ET-770 and **ET-743** were prepared following the same procedures than those previously described in PCT/GB00/01852.

Route 5

The first step of this Route (transformation of **21** into **146**) was described above in Example 71.

Example 90



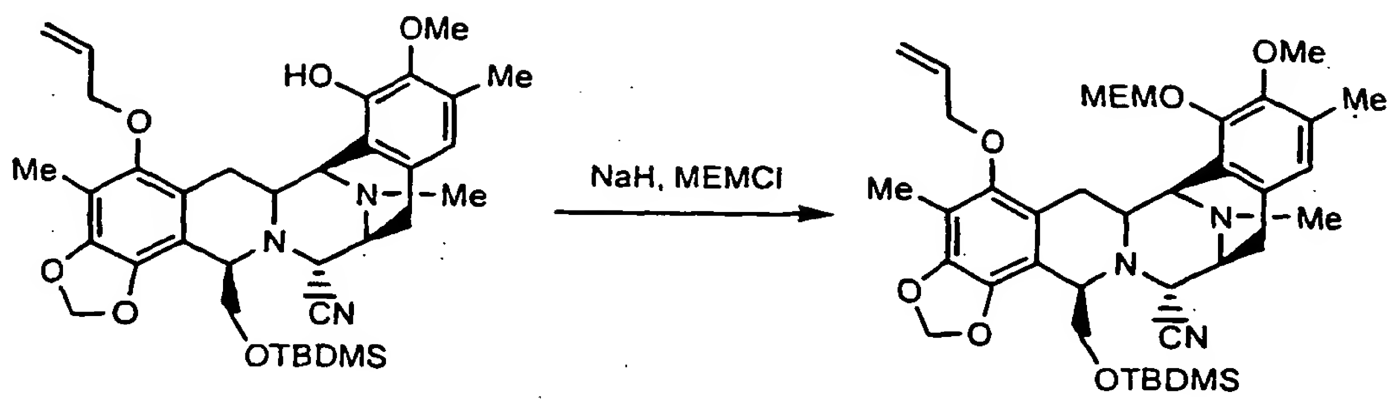
To a solution of **146** (8.72 g, 16.78 mmol) in DMF (20.1 mL), imidazol (3.43 g, 50.34 mmol), *tert*-butyl dimethyl chlorosilane (7.58 mL, 50.34 mmol) and DMAP (0.2 g, 1.7 mmol) were added at 0°C. After being stirred at 23°C for 3.5 h, the reaction mixture was quenched with water (100 mL) and extracted with EtOAc/Hex 1:3 (2 x 75 mL). The combined organic phases were washed with 0.1 M HCl (50 mL) and the aqueous phase was extracted again with EtOAc/Hex 1:3 (40 mL). The combined organic phases were dried over sodium sulphate, filtered and concentrated *in vacuo*. The residue was purified by flash column chromatography (Hex:EtOAc 10:1, 3:1) to obtain **165** (9.85 g, 93%) as a white solid. Rf= 0.39 in Hex:AcOEt 2:1.

^{1}H NMR (300 MHz, $CDCl_3$) □ 6.43 (s,1H), 6.15-6.03 (m, 1H), 5.92 (d, J= 1.2 Hz, 1H), 5.84 (d, J= 1.2 Hz, 1H), 5.67 (s, 1H), 5.41 (dd, J_1 = 1.5, J_2 = 17.1 Hz, 1H), 5.26 (dd, J_1 = 1.5, J_2 = 10.5 Hz, 1H), 4.44 (d, J = 2.7 Hz, 1H), 4.20-4.08 (m, 3H), 3.97 (dd, J_1 = 2.7, J_2 = 8.1 Hz, 1H), 3.75 (s, 3H), 3.61 (dd, J_1 = 2.71, J_2 = 9.9 Hz, 1H), 3.18 (brd, J = 8.7 Hz, 1H), 3.22-3.16 (m, 2H), 2.99 (dd, J_1 = 8.1, J_2= 17.4 Hz, 1H), 2.65 (d, J = 17.4 Hz, 1 H), 2.28 (s, 3H), 2.25 (s, 3H), 2.11 (s, 3H), 1.89 (dd, J_1 = 12, J_2= 15.6 Hz, 1H), 0.8 (s, 9H), -0.05 (s, 3H), -0.09 (s. 3H).

^{13}C NMR (75 MHz, $CDCl_3$) □ 148.2, 146.5, 143.8, 142.4, 138.9, 133.8, 131.0, 128.0, 121.5, 120.4, 118.4, 117.1, 112.8, 111.6, 100.7, 74.0, 68.2, 61.5, 60.2, 58.6, 57.1, 56.5, 55.2, 41.3, 26.2, 25.4, 25.2, 20.6, 17.8, 15.3, 13.8, 9.0, -3.9, -6.0.

ESI-MS m/z: Calcd. for $C_{35}H_{47}N_3O_6Si$: 633.85. Found $(M+1)^+$: 634.2.

Example 91



165 166

To a solution of **165** (7.62 g, 12.02 mmol) in THF (87.64 mL) and water (0.24 mL), MEMCl (2.33 mL, 20.43 mmol) was added at –6°C. After addition of 60% NaH (0.72 g, 18.03 mmol) in portions over 45 min, the mixture was stirred for 1.5 h at that temperature. The reaction was quenched with water (150 mL) and extracted with CH_2Cl_2 (2 x 100 mL). The combined organic phases were dried over Na_2SO_4, filtered and concentrated *in vacuo* to give **166** (8.69 g, 100%) as a white solid which was used in following steps with no further purification. Rf= 0.24 Hex: AcOEt 2:1.

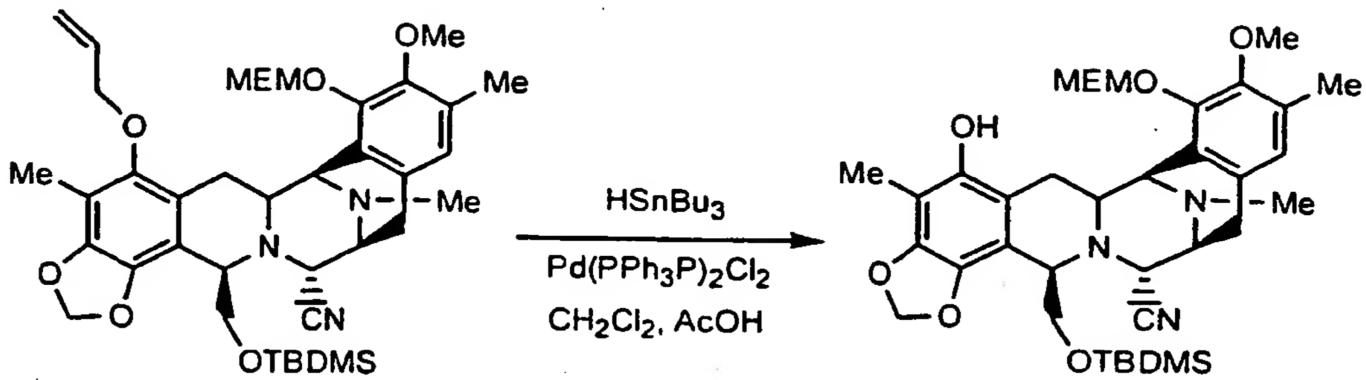
^1H NMR (300 MHz, $CDCl_3$) δ 6.64 (s, 1H), 6.16-6.05 (m,1H), 5.92 (d, J= 1.2 Hz, 1H), 5.85 (d, J= 1.2 Hz, 1H), 5.41 (dd, J_1 = 1.51, J_2 = 17.1 Hz, 1H), 5.29-5.24 (m, 2H), 5.14 (d, J= 6Hz, 1H), 4.42 (d, J= 2.7 Hz, 1H), 4.21-4.06 (m, 3H), 4.01-3.95 (m, 2H), 3.88-3.82 (m, 1H), 3.72 (s, 3H), 3.64-3.57 (m, 3H), 3.39 (s, 3H), 3.29 (brd J= 7.5 Hz, 1H), 3.25-3.15 (m, 2H), 3.00 (dd, J_1 = 8.1, J_2 = 17.4 Hz, 1H), 2.65 (d, J= 18 Hz, 1H), 2.30 (s, 3H), 2.21 (s, 3H), 2.11 (s, 3H), 1.82 (dd J_1 = 12, J_2 = 15.6 Hz, 1H), 0.79 (s, 9H), -0.06 (s, 3H), -0.11 (s, 3H).

^{13}C NMR (75 MHz, $CDCl_3$) δ 148.4, 148.1, 144.1, 139.2, 133.9, 130.9, 130.8, 130.2, 128.8, 125.1, 124.2, 121.5, 118.8, 117.45, 113.0, 111.9,

101.0, 98.2, 74.1, 71.7, 69.3, 68.3, 61.7, 59.6, 59.0, 58.9, 57.3, 57.1, 55.5, 41.6, 29.7, 26.4, 25.8, 25.5, 25.4, 15.7, 9.2, -5.6, -5.6.

ESI-MS m/z: Calcd. for $C_{39}H_{55}N_3O_8Si$: 721.3. Found $(M+1)^+$: 722.3.

Example 92



166 **167**

To a solution of **166** (10.76 g, 14.90 mmol) in anhydrous CH_2Cl_2 (275 mL), $Pd(PPh_3P)_2Cl_2$ (837 mg, 1.19 mmol), acetic acid (4.26 mL, 74.5 mmol) and tributyltin hydride (11.85 mL, 44.7 mmol) were added under Argon atmosphere at 23 °C. The reaction mixture was stirred at 23 °C for 15 minutes. (TLC AcOEt/Hexane 1:1 showed no starting material). Hexane (100 mL) was added and the mixture was poured into a flash column chromatography, (SiO_2, EtOAc:Hexane in a gradient manner, from 0:100, 1:4, 2:3 to 1:1) to afford **167** (9.95 g, 98%) as a yellow solid. Rf = 0.42 Hex:EtOAc 3:7.

^{1}H-RMN (300 MHz, $CDCl_3$): δ 6.63 (s, 1H), 5.89 (d, J = 1.4 Hz, 1H), 5.79 (d, J = 1.4 Hz, 1H), 5.76 (m, 1H), 5.38 (d, J = 5.6 Hz, 1H), 5.23 (d, J = 5.9 Hz, 1H), 4.53 (d, J = 2.7 Hz, 1H), 4.17 (dd, J_1 = 1.95 Hz, J_2 = 6.05 Hz, 1H), 4.11 (dd, J_1 = 7.0 Hz, J_2 = 12.5 Hz, 1H), 4.01-3.92 (m, 2H), 3.70 (s, 3H), 3.67 (m, 3H), 3.40 (s, 3H), 3.29 (m, 1H), 3.24-3.13 (m, 3H), 2.99 (dd, J_1 = 8.0 Hz J_2 = 17.5 Hz, 1H), 2.67 (d, J = 17.5 Hz, 1H), 2.28

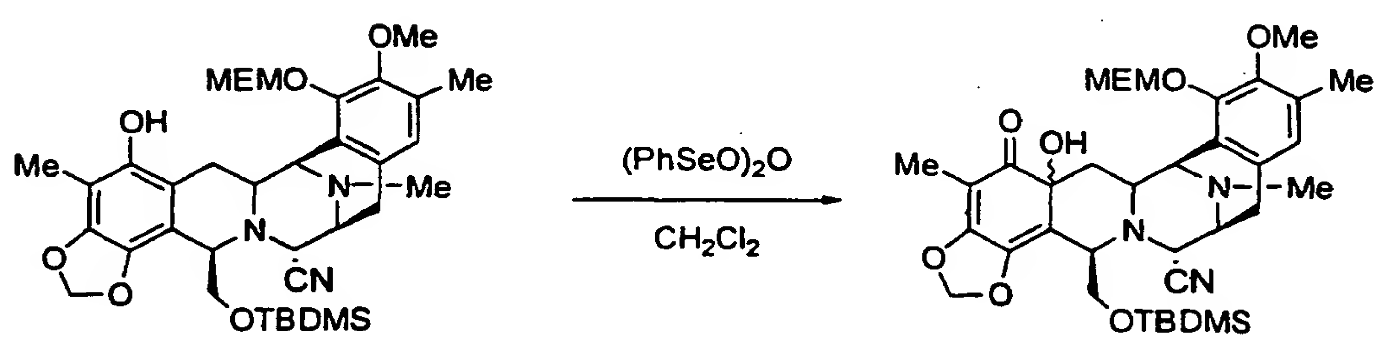
(s, 3H), 2.09 (s, 3H), 2.05 (s, 3H), 1.80 (dd, J_1 = 11.2 Hz, J_2 = 14.9 Hz, 1H), 0.82 (s, 9H), -0.03 (s, 3H), -0.07 (s, 3H).

^{13}C-RMN (75 MHz, $CDCl_3$): δ 148.4, 147.3, 145.5, 144.1, 136.2, 134.9, 134.8, 130.9, 130.2, 124.8, 123.1, 118.6, 112.8, 112.1, 106.2, 100.4, 98.4, 71.5, 69.2, 68.9, 61.7, 59.6, 58.7, 58.6, 56.9, 56.6, 55.3, 41.5, 29.5, 25.7, 25.3, 17.9, 15.5, 8.7, -5.7, -5.8.

ESI-MS m/z: Calcd. for $C_{36}H_{51}N_3O_8Si$: 681.89. Found $(M+1)^+$: 682.3.

HPLC: Conditions: Column: Symmetry C18; mobile phase: AcN - buffer phosphate 25mM, pH=5, isocratic of AcN (65%) in 5 minutes and gradient in AcN from 65-92% in 31 minutes, Ø: 0.6 mL/min, t^a: 40 °C. Retention time: 27.89 minutes. HPLC purity in area: 89.62%.

Example 93



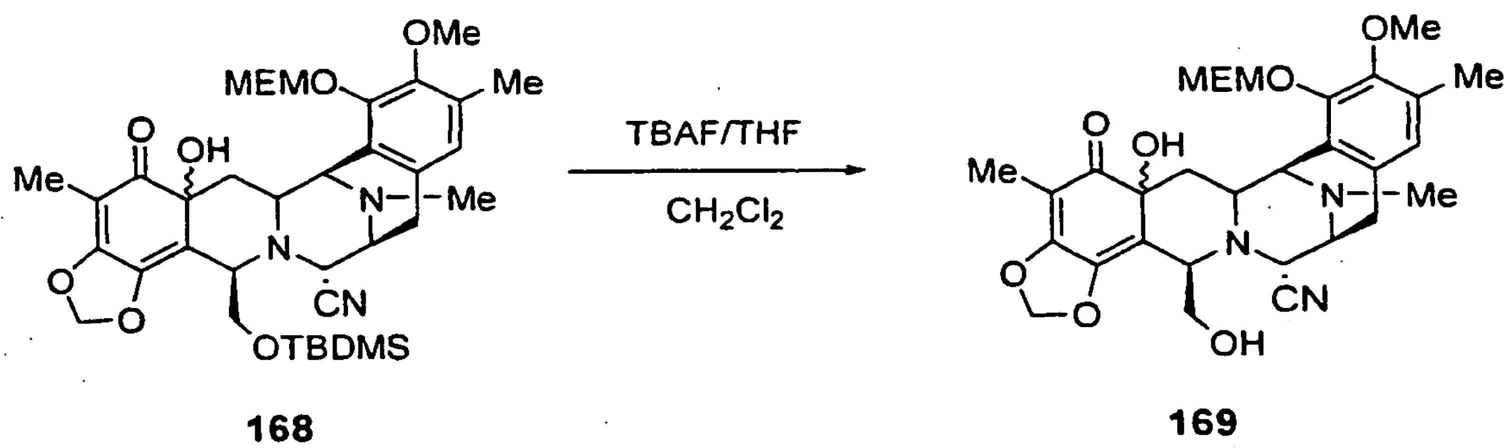
167 168

To a solution of **167** (9.95 g, 14.6 mmol) in anhydrous CH_2Cl_2 (300 mL), a solution of benceneseleninic anhydride (7.51 g, 14.6 mmol, reagent purity 70%) in anhydrous CH_2Cl_2 (120 mL) was dropwise added, under Argon atmosphere at −15 °C (the remaning white solid was discarded). The solution was then stirred at −15 °C for 15 minutes (TLC EtOAc/Hexane 2:3, showed no starting material). A saturated aqueous solution of sodium bicarbonate (500 mL) was added to the reaction mixture at this temperature. The organic phase was separated and the aqueous phase was extracted with CH_2Cl_2 (500 mL). The combined organic extracts were dried over sodium sulphate, filtered and the solvent was eliminated under reduced pressure. The crude of the reaction was purified by flash column chromatography, (SiO_2, EtOAc:Hexane in a gradient manner, from 2:3 to 3:1) to afford **168** (9.86 g, 97%) as a yellow solid. Rf = 0.33 Hex:EtOAc 3:7).

^{1}H-RMN (300 MHz, $CDCl_3$) (Isomers ratio: ≈ 3:2): δ 6.59 (s, 1H), 6.57 (s, 1H), 5.77 (s, 1H), 5.76 (s, 1H), 5.68 (s, 1H), 5.63 (s, 1H), 5.19 (d, J = 6.0 Hz, 1H), 5.09 (d, J = 6.0 Hz, 1H), 5.07 (d, J = 6.1 Hz, 1H), 5.00 (d, J = 6.1 Hz, 1H), 4.40 (d, J = 2.7 Hz, 1H), 4.27 (d, J = 2.44 Hz, 1H), 4.22 (d, J = 10.5 Hz, 1H), 3.95 (d, J = 1.7 Hz, 1H), 3.86-3.75 (m, 2H), 3.81 (s, 3H), 3.72-3.68 (m, 2H), 3.65 (m, 2H), 3.54 (s, 3H), 3.50 (m, 3H), 3.31 (s, 3H), 3.29 (s, 3H); 3.24 (m, 1H), 3.09 (dt, J = 3.2 Hz, J = 7.6 Hz, 1H), 3.02 (d, J = 11.2 Hz, 1H), 2.92 (m, 2H), 2.48 (d, J = 9.5 Hz, 1H), 2.43 (d, J = 9.3 Hz, 1H), 2.21 (s, 3H), 2.14 (s, 3H), 2.13 (s, 3H), 2.03 (m, 2H), 1.73 (s, 3H), 1.71 (s, 3H), 0.86 (s, 9H), 0.77 (s, 9H), 0.04 (s, 3H), 0.02 (s, 3H). ^{13}C-RMN (75 MHz, $CDCl_3$): 200.5, 197.2, 159.8, 157.7, 148.4, 148.2, 147.7, 140.0, 137.6, 130.5, 130.2, 129.9, 129.4, 124.9, 124.7, 124.0, 122.7, 117.1, 116.9, 113.4, 110.8, 103.9, 103.8, 101.0, 100.4, 97.8, 72.8, 71.3, 69.7, 68.9, 68.8, 65.4, 64.1, 60.2, 59.9, 59.3, 59.1, 59.0, 58.6, 58.5, 56.8, 56.5, 56.2, 55.5, 54.9, 54.8, 42.5, 41.1, 40.9, 35.8, 25.6, 25.5, 25.4, 25.3, 20.6, 17.9, 17.8, 15.5, 15.3, 13.8, 7.0, 6.7, -5.7, -6.0, -6.1.

ESI-MS m/z: Calcd. for $C_{36}H_{51}N_3O_9Si$: 697.89. Found $(M+1)^+$: 698.8
HPLC: Conditions: Column: Symmetry C18; mobile phase: AcN, buffer phosphate 25mM, pH=5, gradient in AcN from 30-100% in 50 minutes. Ø: 1.2 mL/min, t[a]: 40 °C. Retention time: 30.70 minutes and 30.95 minutes (the two isomers). HPLC purity in area: 60.77% and 31.99%.

Example 94



To a solution of **168** (16.38 g, 23.47 mmol) in anhydrous THF (727 mL, 0.03 M), a solution of TBAF in 1M THF (59 mL, 59 mmol) was dropwise added at 23°C. The reaction mixture was stirred at 23 °C for 45 minutes. Then, the mixture was partitioned between a saturated aqueous NaCl solution (850 mL) and CH_2Cl_2 (950 mL). Both layers were separated and the organic layer was dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by flash column chromatography (SiO_2, EtOAc:Hexane in a gradient manner, from 40:60, 50:50, 70:30, 90:10 to 100:0) to afford **169** (12.17 g, 89%) as a light yellow solid. Rf = 0.1 Hex:EtOAc 3:7.

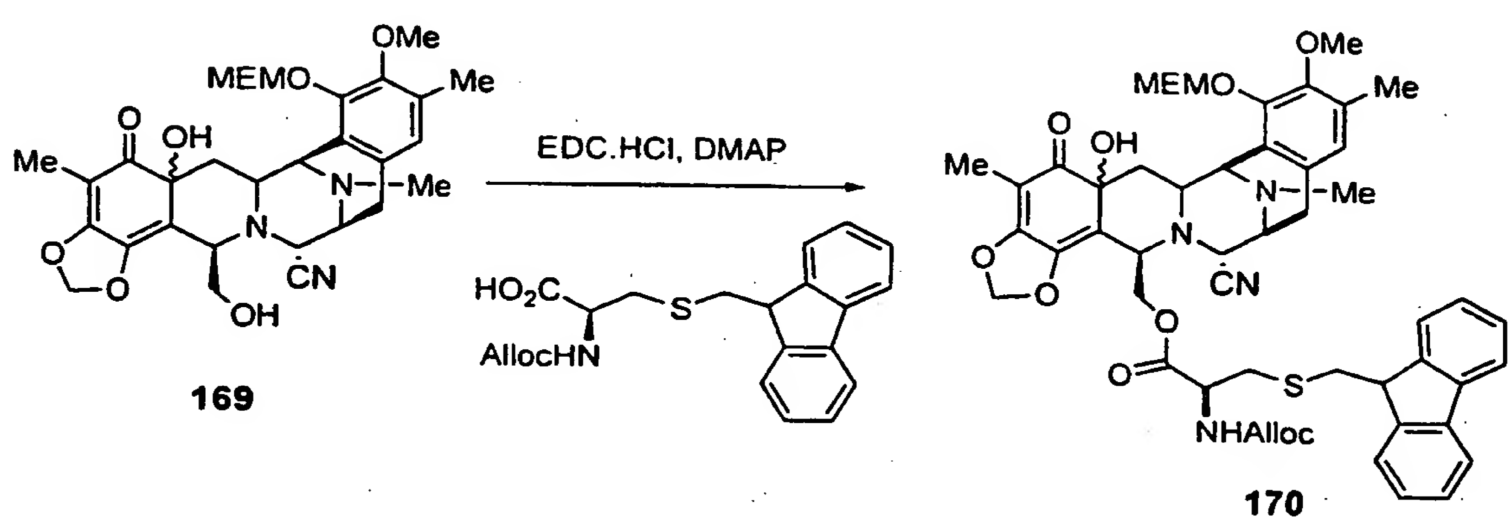
^{1}H-RMN (300 MHz, $CDCl_3$) (Isomers ratio: 3:2): δ 6.63 (s, 1H), 6.57 (s, 1H), 5.79 (s, 1H), 5.77 (s, 1H), 5.75 (s, 1H), 5.62 (s, 1H), 5.23 (s, 1H), 5.18 (d, J = 6.1 Hz, 1H), 5.08 (d, J = 6.1 Hz, 1H), 5.01 (d, J = 6.1 Hz, 1H), 4.22 (d, J = 2.7 Hz, 1H), 4.09 (d, J = 2.4 Hz, 1H), 4.00 (m, 4H), 3.82 (s, 3H), 3.87-3.64 (m, 6H), 3.55 (s, 3H), 3.51-3.44 (m, 2H), 3.30 (s, 3H), 3.29 (s, 3H), 3.26 (m, 1H), 3.18 (dt, J_1 = 2.9 Hz, J_2 = 7.3 Hz, 1H), 2.94

(m, 4H) 2.50 (m, 4H), 2.22 (s, 3H), 2.16 (s, 3H), 2.15 (s, 3H), 2.11 (s, 3H), 2.02 (d, J = 7.3 Hz, 2H), 1.72 (s, 3H), 1.69 (s, 3H).

^{13}C-RMN (75 MHz, $CDCl_3$): 200.2, 200.1, 159.6, 158.5, 148.5, 148.4, 148.1, 147.9, 140.5, 137.4, 130.9, 130.4, 130.1, 130.0, 125.1, 124.9, 123.8, 122.7, 116.9, 116.6, 113.3, 110.7, 104.5, 103.9, 101.4, 100.7, 98.1, 97.9, 71.9, 71.5, 71.4, 70.1, 69.0, 69.0, 62.0, 60.1, 59.5, 58.7, 58.5, 58.1, 57.4, 56.9, 56.8, 56.4, 55.9, 55.1, 55.0, 41.3, 41.0, 36.1, 31.3, 25.3, 25.2, 22.4, 15.6, 15.5, 13.8, 7.0, 6.8.

ESI-MS m/z: Calcd. for $C_{30}H_{37}N_3O_9$: 583.63. Found $(M+1)^+$: 584.2.

Example 95

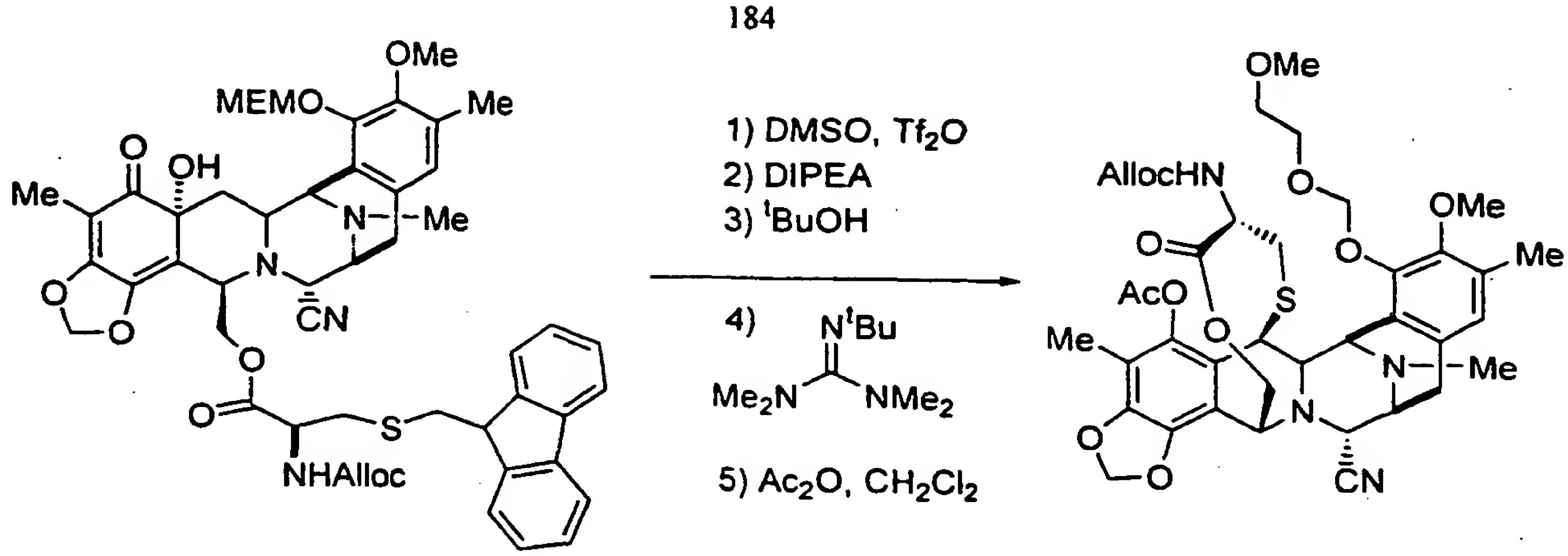


To a solution of **169** (11.49 g, 19.69 mmol) and Alloc-Cys-(Fm) (11.32 g, 29.53 mmol) (for its preparation see Kruse, C. H.; Holden, K. G., *J. Org. Chem.*, 1985, 50, pp. 2792-2794) in anhydrous CH_2Cl_2 (688 mL), DMAP (2.4 g, 19.69 mmol) and EDC·HCl (9.44 g, 49.22 mmol) were added at 23°C. Then, DIPEA (5.14 mL, 29.53 mmol) was added at 0°C and the reaction was stirred at 23°C for 3 hour. The mixture was washed successively with a saturated aqueous solution of $NaHCO_3$ (500 mL),

NaCl (400 mL) and NH_4Cl (2 x 300 mL). The organic layer was dried over Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by flash column chromatography (SiO_2, AcOEt:Hex in a gradient manner, from 1:1, 6:4 to 7:3) to afford **170** (14.76 g, 79%) as a pale yellow solid. Rf = 0.31 and 0.40 Hex:EtOAc 3:7 (mixture of isomers).

^{1}H-RMN (300 MHz, $CDCl_3$): 7.74 (d, J = 7.6 Hz, 4H), 7.63 (dd, J = 7.0 Hz, J = 15.3 Hz, 4H), 7.38 (t, J = 7.3 Hz, 4H), 7.29 (m, 4H), 6.61 (s, 1H), 6.54 (s, 1H), 5.89 (m, 2H); 5.73 (s, 1H), 5.70 (s, 1H), 5.69 (s, 1H), 5.62 (s, 1H), 5.55 (m, 1H), 5.32 (d, J = 15.1 Hz, 1H), 5.23 (d, J = 6.1 Hz, 1H), 5.22 (d, J = 10.6 Hz, 1H), 5.14 (d, J = 5.9 Hz, 1H), 5.13 (d, J =6.0 Hz, 1H), 5.07 (d, J = 6.3 Hz, 1H), 4.68 (m, 1H), 4.56 (m, 4H), 4.51 (m, 2H), 4.38 (dd, J_1 = 4.5 Hz, J_2 = 12.6 Hz, 1H), 4.22 (dd, J_1 = 6.2 Hz, J_2 = 11.1 Hz, 1H), 4.14-3.88 (m, 12H), 3.83 (s, 3H), 3.79-3.69 (m, 4H), 3.61 (s, 3H), 3.56 (m, 4H), 3.39 (s, 3H), 3.36 (s, 3H), 3.23 (m, 2H), 3.16 (d, J = 6.0 Hz, 2H), 3.07 (d, J = 6.1 Hz, 2H), 3.00- 2.81 (m, 6H), 2.46-2.34 (m, 4H), 2.25 (s, 3H), 2.20 (s, 3H), 2.16 (s, 3H), 2.07 (m, 1H), 1.83 (dd, J_1 = 9.5 Hz, J_2 = 15.1 Hz, 1H), 1.78 (s, 3H), 1.77 (s, 3H).
^{13}C- RMN (75 MHz, $CDCl_3$): δ 200.3, 198.4, 170.3, 160.0, 158.1, 148.7, 148.7, 148,5, 148.2, 145.6, 145.6, 145.5, 142.2, 141.1, 141.0, 141.0, 138.5, 132.4, 132.3, 131.1, 130.6, 130.1, 129.8, 128.8, 127.6, 127.1, 127.1, 125.1, 125.0, 124.8, 124.7, 124.7, 124.0, 122.7, 119.9, 118.1, 118.0, 117.2, 116.8, 111.6, 108.3, 104.8, 104.5, 101.5, 101.0, 98.2, 98.2, 72.3, 71.7, 71.7, 70.6, 69.3, 69.2, 66.4, 66.0, 66.0, 65.5, 63.8, 60.8, 60.2, 59.8, 59.0, 58.9, 58.1, 56.8. 56.6, 56.5, 56.3, 56.1, 55.7, 55.3, 55.2, 53.9, 46.9, 41.9, 41.4, 41.2, 37.2, 36.9, 35.4, 31.5, 29.6, 25.6, 25.4, 22.6, 15.8, 15.7, 14.1, 7.3, 7.0.
ESI-MS m/z: Calcd. for $C_{51}H_{56}N_4O_{12}S$: 948.36. Found $(M+1)^+$: 949.3.

Example 96



170 171

The reaction flask was flamed twice, purged vacuum/Argon several times and kept under Argon atmosphere for the reaction. To a solution of DMSO (5.4 mL) in anhydrous CH_2Cl_2 (554 mL) was dropwise added triflic anhydride (5.11 mL, 30.4 mmol) at –78°C. The reaction mixture was stirred at –78°C for 20 minutes. Then, a solution of **170** (14.43 g, 15.2 mmol) in anhydrous CH_2Cl_2 (188 mL) at –78°C was added *via* canula. During the addition the temperature was kept at –78°C in both flasks and the color of the reaction was yellow. The reaction mixture was stirred at –40°C for 35 minutes. During this period of time the solution was turned from yellow to dark green. After this time, $^iPr_2NEt$ (21.2 mL, 121.6 mmol) was dropwise added and the reaction mixture was kept at 0°C for 45 minutes. The color of the solution turned to pale brown during this time. Then, ^tBuOH (5.8 mL, 60.8 mmol) and *tert*-butyl tetramethyl guanidine (18.3 mL, 106.4 mmol) were dropwise added and the reaction mixture was stirred at 23°C for 40 minutes. After this time, acetic anhydride (14.34 mL, 152 mmol) was dropwise added and the reaction mixture was kept at 23°C for 1 hour more. Then, the reaction mixture was diluted with CH_2Cl_2 (38 mL) and washed with a saturated aqueous solution of NH_4Cl (500 mL), $NaHCO_3$ (500 mL), and NaCl (500 mL). The combined organic layers were dried over Na_2SO_4, filtered and concentrated. The residue was purified by flash column chromatography (SiO_2, EtOAc:Hex in a gradient manner, from

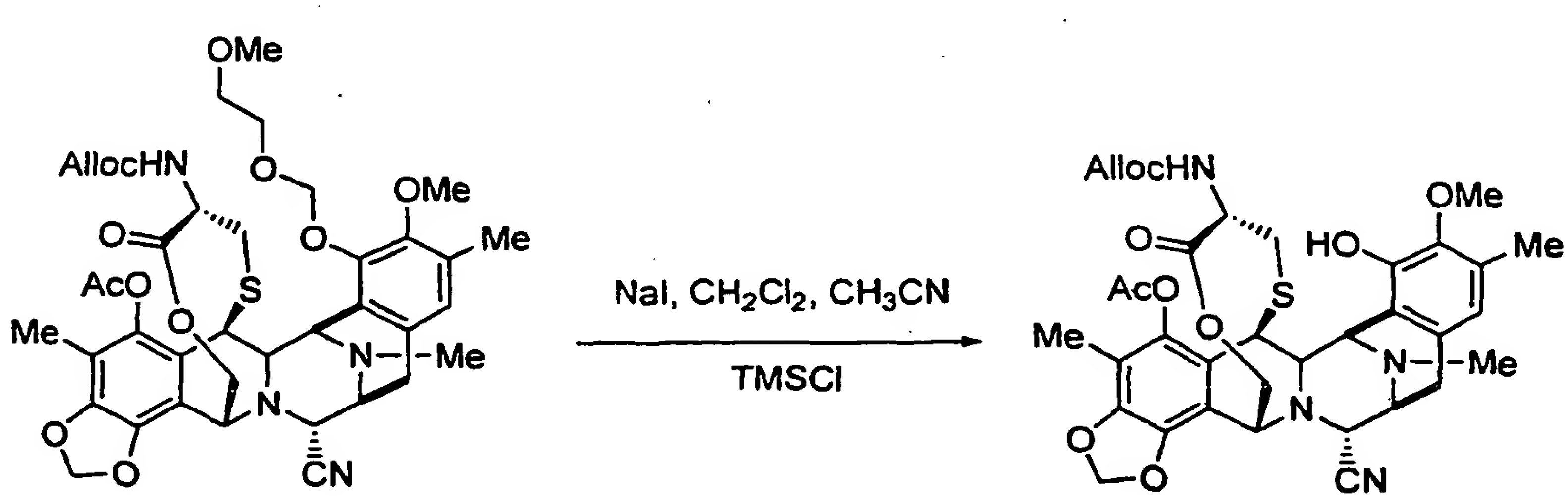
3:7 to 4:6) to afford **171** (6.24 g, 52%) as a pale yellow solid. Rf = 0.38 Hex:EtOAc 1:1.

^{1}H-RMN ($CDCl_3$): 6.78 (s, 1H), 6.07 (d, J = 1.2 Hz, 1H), 5.98 (d, J = 1.2 Hz, 1H), 5.92 (m, 1H), 5.32 (d, J = 5.9 Hz, 1H), 5.31 (dd, J_1 = 1.5 Hz, J_2 = 17.1 Hz, 1H), 5.23 (dd, J_1 = 1.5 Hz, J_2 = 10.4 Hz, 1H), 5.19 (d, J = 5.6 Hz, 1H), 5.01 (d, J = 11.5 Hz, 1H), 4.81 (d, J = 9.8 Hz, 1H), 4.53-4.51 (m, 3H), 4.35-4.27 (m, 2H), 4.24 (s, 1H), 4.18-4.13 (m, 2H), 3.94-3.84 (m, 2H), 3.73 (s, 3H), 3.58 (t, J = 4.7 Hz, 2H), 3.43-3.37 (m, 2H), 3.36 (s, 3H), 2.91 (m, 2H), 2.27 (s, 3H), 2.26 (s, 3H), 2.20 (s, 3H), 2.36-2.06 (m, 2H), 2.02 (s, 3H).

^{13}C-RMN ($CDCl_3$): 170.23, 168.49, 155.26, 149.62, 148.26, 145.63, 140.85, 140.24, 132.74, 131.60, 130.11, 124.89, 124.70, 120.14, 117.89, 117.84, 113.21, 101.89, 98.03, 92.67, 71.60, 69.04, 65.70, 61.20, 60.35, 59.36, 59.01, 58.89, 54.71, 54.42, 53.79, 41.53, 41.19, 32.68, 29.53, 23.57, 20.26, 15.62, 9.45.

ESI-MS m/z: Calcd. for $C_{39}H_{46}N_4O_{12}S$: 794.87. Found: 796 $(M+1)^+$, 817 $(M+23)^+$. HPLC: Conditions: Column: Simmetry C18, Mobile phase: AcN/buffer phosphate (pH: 5) in gradient from 45 to 65% in 15 minutes and 65-90% in 36 minutes. ∅ = 0.8 ml/min, t^a= 40 °C. Retention time: 19.734 minutes. HPLC purity in area: 83.17%

Example 97



171 164

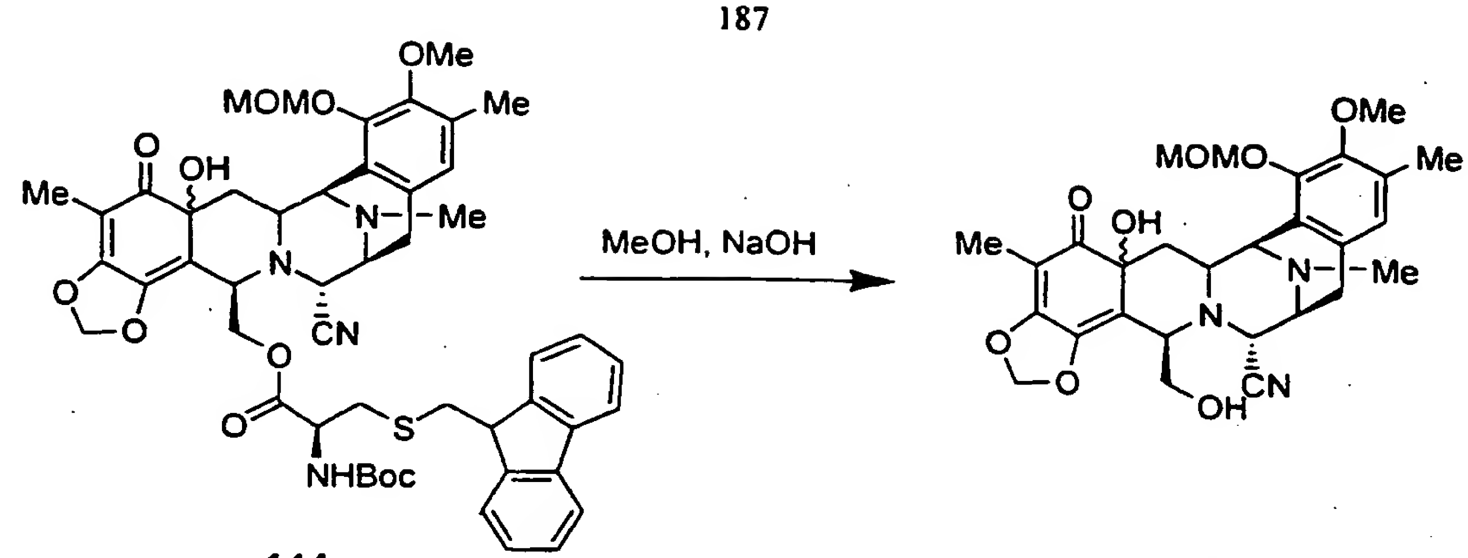
To a solution of **171** (2.26 g, 2.85 mmol) in anhydrous CH_2Cl_2 (74 mL) and acetonitrile (74 mL), NaI (3.42 g, 22.8 mmol) and TMSCl (freshly distilled over CaH_2) (2.6 mL, 22.8 mmol) were added at 0°C and the reaction was stirred for 35 minutes. A saturated aqueous solution of sodium bicarbonate (150 mL) was added to the reaction mixture at this temperature. The organic phase was separated and the aqueous phase was extracted with CH_2Cl_2 (2 x 100 mL). The combined organic extracts were dried over sodium sulphate, filtered and the solvent was eliminated under reduced pressure to give **164** (2.4 g, 100%) as a pale yellow solid which was used in subsequent reactions with no further purification. Experimental data of **164** were described above in Example 88.

Transformation of **164** into **35** was previously described above in Example 89.

Intermediates **35**, **36**, **ET-770** and **ET-743** were prepared following the same procedures than those previously described in PCT/GB00/01852.

Route 6

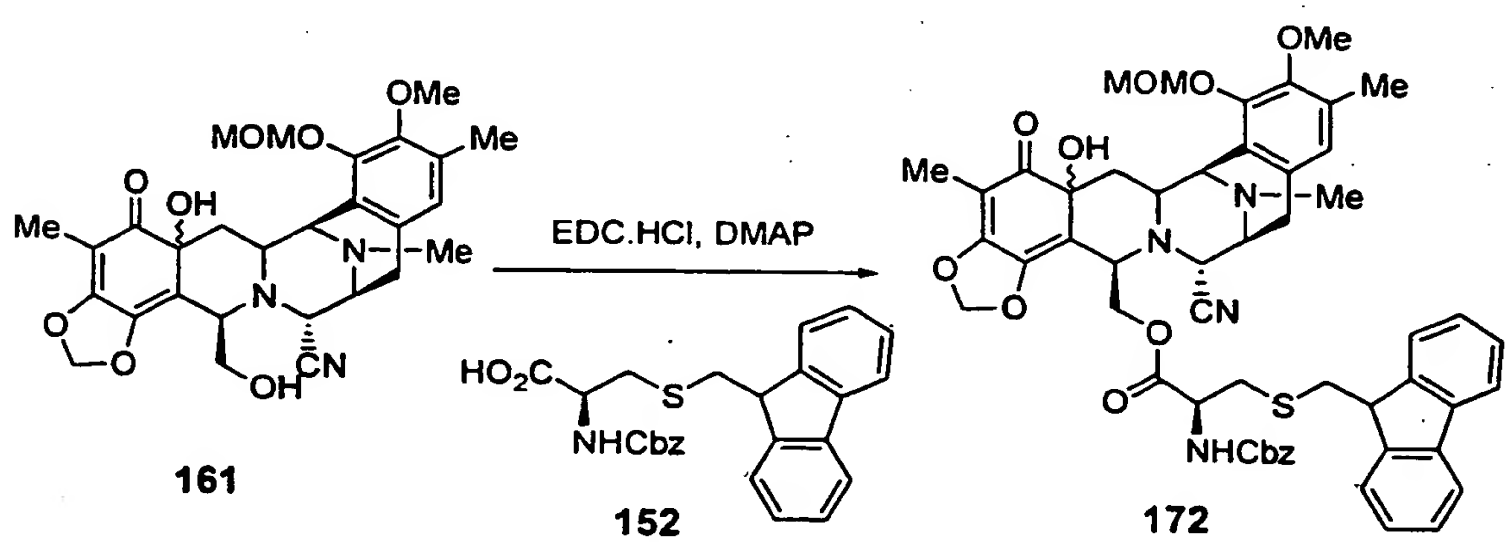
Example 98



144 161

To a solution of **144** (7 g, 7.6 mmol) in MeOH (140 mL), 1M NaOH (15.1 mL) was added and the reaction was stirred for 10 minutes at 23°C. A saturated aqueous solution of NH_4Cl (100 mL) was added to the reaction mixture. The organic phase was separated and washed with 5% HCl until the colour turned into yellow. The organic extract was dried over sodium sulphate, filtered and the solvent was eliminated under reduced pressure. The residue was purified by flash column chromatography (SiO_2, EtOAc:Hexane in a gradient manner, from 0:1, 1:3, 1:2, 1:1, 1:1 to 3:1) to afford **161** (3.76 g, 85%). Experimental data of **161** were previously described in U. S. Patent No 5,721,362.

Example 99



161 152 172

To a solution of **161** (200 mg, 0.37 mmol) and the cysteine **152** (240 mg, 0.55 mmol) in anhydrous CH_2Cl_2 (20 mL), DMAP (110 mg, 0.925

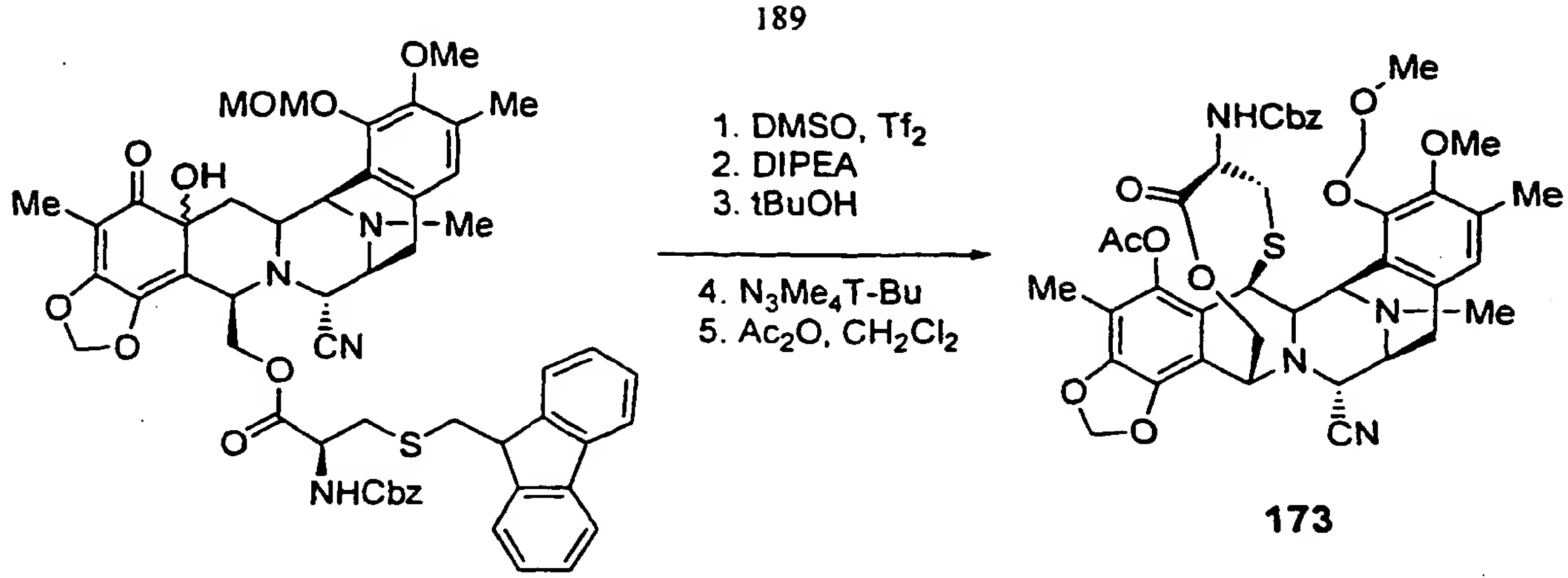
mmol) and EDC·HCl (170 mg, 0.925 mmol) were added at 23°C and the reaction was stirred at that temperature for 1.5 hours. The mixture was then washed successively with a saturated aqueous solution of $NaHCO_3$ (15 mL), NaCl (15 mL) and NH_4Cl (2 x 10 mL). The organic layer was dried over Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by flash column chromatography with silica gel (SiO_2, AcOEt/Hexane in a gradient manner, from 1:4 to 1:2) to afford **172** (285 mg, 80%) as a white solid. Rf = 0.3 Hex:EtOAc 2:1.

^{1}H RMN ($CDCl_3$) δ 7.73 (d, J = 7.5 Hz, 2H), 7.59-7.57 (m, 2H), 7.40-7.28 (m, 9H), 6.60 (s, 1H), 5.69 (s, 1H), 5.65 (s, 1H), 5.54 (d, J = 7.8 Hz, 1H), 5.11-5.08 (m, 4H), 4.52-4.49 (m, 1H), 4.21-3.90 (m, 6H), 3.83 (s, 3H), 3.49 (s, 3H), 3.21 (d, J = 6.6 Hz, 1H), 3.09-2.90 (m, 6H), 2.41 (d, J = 18 Hz, 1H), 2.34-2.31 (m, 1H), 2.25 (s, 3H), 2.19 (s, 3H), 1.88-1.83 (m, 1H), 1.77 (s, 3H).

^{13}C-RMN ($CDCl_3$) δ 198.7, 170.5, 158.4, 155.9, 148.9, 148.8, 145.8, 142.5, 141.3, 136.2, 131.4, 130.0, 128.8, 128.6, 128.4, 127.9, 127.3, 125.3, 125.0, 124.9, 123.0, 120.1, 117.5, 108.5, 104.8, 101.7, 99.5, 70.8, 67.4, 60.5, 57.8, 57.0, 56.5, 56.0, 55.5, 47.1, 41.6, 37.4, 37.1, 31.8, 25.8, 22.8, 15.9, 14.3, 7.6.

ESI-MS m/z: Calcd. for $C_{53}H_{54}N_4O_{11}S$: 954.35. Found $(M+23)^+$: 977.8.

Example 100



172

173

The reaction flask was flamed twice, purged vacuum/Argon several times and kept under Argon atmosphere for the reaction. To a solution of DMSO (977 μL) in anhydrous CH_2Cl_2 (118 mL) was dropwise added triflic anhydride (930 □L, 5.5 mmol) at -78 °C. The reaction mixture was stirred at -78 °C for 20 minutes. Then, a solution of **172** (2.63 g, 2.75 mmol) in anhydrous CH_2Cl_2 (26 mL, for the main addition and 13 mL for washing) was added *via* canula (addition time: 5 min) at -78 °C. During the addition the temperature was kept at -78 °C in both flasks and the color changed from yellow to brown. The reaction mixture was stirred at -40 °C for 35 minutes. During this period of time the solution was turned from yellow to dark green. After this time, $^iPr_2NEt$ (3.48 mL, 22 mmol) was dropwise added and the reaction mixture was kept at 0 °C for 45 minutes, the color of the solution turned brown during this time. Then, ^tBuOH (1.04 mL, 11 mmol) and *tert*-butyl tetramethyl guanidine (3.31 mL, 19.25 mmol) were dropwise added and the reaction mixture was stirred at 23 °C for 40 minutes. After this time, acetic anhydride (2.6 mL, 27.5 mmol) was dropwise added and the reaction mixture was kept at 23 °C for 1 hour more. Then, the reaction mixture was diluted with CH_2Cl_2 (70 mL) and washed successively with a saturated aqueous solution of NH_4Cl (180 mL), $NaHCO_3$ (180 mL), and NaCl (180 mL). The combined organic layers were dried over Na_2SO_4, filtered and concentrated at reduced pressure. The residue was purified by flash column chromatography (SiO_2, Hex:EtOAc in a gradient

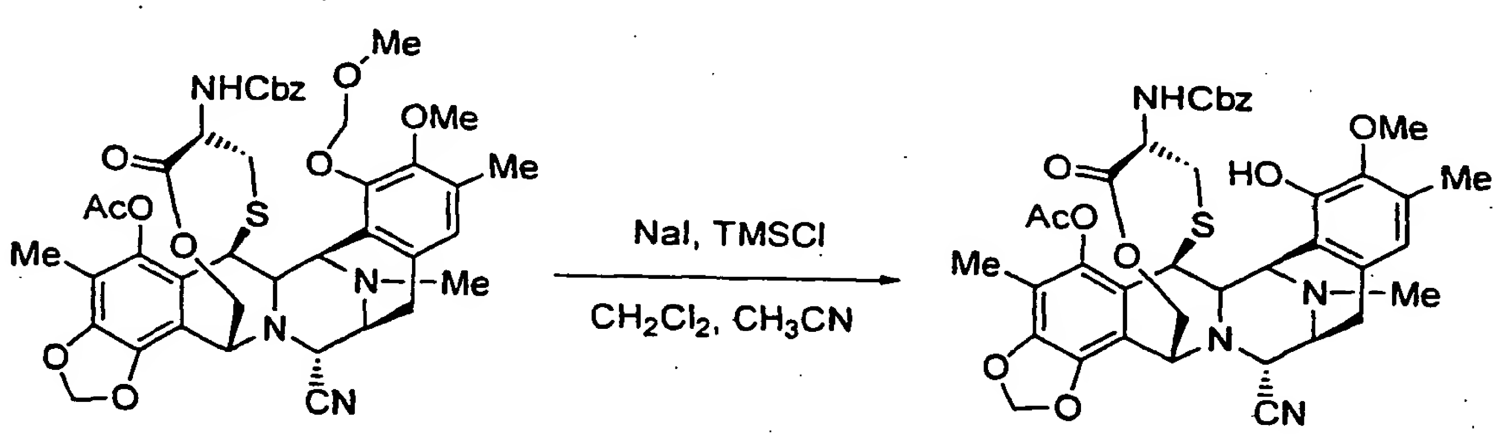
manner, from 4:1, 3:1, to 2:1) to afford **173** (1.145 g, 52%) as a white solid. Rf = 0.31 Hex:EtOAc 3:2.

^{1}H RMN ($CDCl_3$) δ 7.37 (bs, 5H), 6.67 (s, 1H), 6.08 (d, J = 1.2 Hz, 1H), 5.99 (d, J = 1.2 Hz, 1H), 5.19-5.00 (m, 4H), 4.82 (d, J = 9.3 Hz, 1H), 4.49 (bs, 1H), 4.32-4.15 (m, 5H), 3.67 (s, 3H), 3.55 (s, 3H), 3.44 (d, J = 4.8 Hz, 1H), 3.39 (d, J = 6 Hz, 1H), 2.90-2.87 (m, 2H), 2.28 (s, 3H), 2.19 (s, 3H), 2.15-2.07 (m, 2H), 2.03 (s, 3H), 2.00 (s, 3H).

^{13}C-RMN ($CDCl_3$) δ 170.6, 168.8, 155.8, 149.9, 148.5, 146.0, 141.2, 140.6, 136.6, 132.0, 130.4, 128.8, 128.7, 128.5, 125.2, 124.9, 120.5, 118.2, 113.7, 113.6, 102.2, 99.4, 67.2, 61.6, 60.7, 59.7, 59.3, 57.6, 55.1, 54.8, 54.2, 41.9, 41.6, 33.0, 29.9, 23.9, 20.6, 15.6, 9.8.

ESI-MS m/z: Calcd. for $C_{41}H_{44}N_4O_{11}S$: 800.87. Found $(M+23)^+$: 823.7.

Example 101



173 158

To a solution of **173** (100 mg, 0.125 mmol) in CH_2Cl_2 (2 mL) and CH_3CN (2 mL), NaI (75 mg, 0.5 mmol) and TMSCl (63 □L, 0.5 mmol) were added at 0°C. After stirring the reaction at 23°C for 50 minutes, the mixture was quenched with water (30 mL) and extracted with CH_2Cl_2 (2 x 20 mL). The combined organic phases were washed successively with a saturated aqueous solution of NaCl (20 mL) and sodium ditionite (20 mL), dried over Na_2SO_4, filtered and concentrated *in vacuo*. The residue

was purified by flash column chromatography (SiO_2, EtOAc:Hexane in a gradient manner, from 1:4, 1:2 to 1:1) to afford **158** (66 mg, 70%) as white solid. Rf = 0.21 Hex:EtOAc 1:1. Experimental data of **158** was described above in Example 19.

Transformation of **158** into **35** was described above in Example 85.

Intermediates **36**, **ET-770** and **ET-743** were prepared following the same procedures than those previously described in PCT/GB00/01852.

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Claims

1. A process for preparing an ecteinascidin product with a spiroamine-1,4-bridge, the process involving forming a 1,4 bridge using a 1-labile, 10-hydroxy, 18-protected hydroxy, di-6,8-en-5-one fused ring compound, wherein C-18 protection is removed before spiroamine introduction.

2. A process according to claim 1, wherein the ecteinascidin product has a 21-hydroxy group, the process including converting a 21-cyano group to the 21-hydroxy group.

3. A process according to claim 1 or 2, wherein the spiroamine is a spiroquinoline.

4. A process according to any preceding claim, wherein the 18-protected group of the 1-labile, 10-hydroxy, 18-protected hydroxy, di-6,8-en-5-one fused ring compound is protected with: MOM, methoxymethyl; or MEM, methoxyethoxymethyl group.

5. A process according to any preceding claim, wherein the 1-labile group is an N-protected cysteinyloxymethylene group of the formula

$$-CH_2\text{-}O\text{-}CO\text{-}CNHProt^1\text{-}CH_2\text{-}S\text{-}H.$$

6. A process according to claim 5, where Prot[1] is: Boc, t-butyloxycarbonyl; Troc, 2,2,2-trichloroethyloxycarbonyl; Cbz, benzyloxycarbonyl; or Alloc, allyloxycarbonyl.

7. A process according to claim 5 or 6, wherein Prot[1] is removed in the same step as C-18 protection.

8. A process according to claim 5, 6 or 7, wherein the 1-labile group is generated from a 1-substituent of the formula:

$$-CH_2-O-CO-CNHProt^1-CH_2-S-Prot^2.$$

9. A process according to claim 8, wherein Prot[2] is Fm, 9-fluorenylmethyl.

10. A process according to claim 8 or 9, wherein the 1-substituent of the formula:

$$-CH_2-O-CO-CNHProt^1-CH_2-S-Prot^2.$$

is formed by esterification of a $-CH_2-O-H$ substituent.

11. A process according to claim 10, wherein the esterification is carried out before formation of the 10-hydroxy, di-6,8-en-5-one structure.

12. A process according to claim 10, wherein the esterification is carried out after introduction of the 10-hydroxy, di-6,8-en-5-one structure.

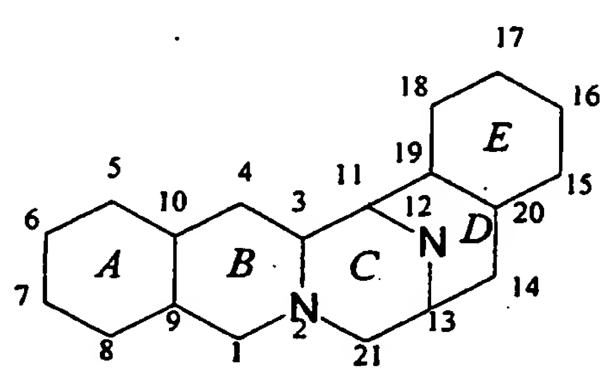
13. A process according to any preceding claim, which starts from a 1-aminomethylene, 5-protected hydroxy, 7,8-dioxymethylene, 18-hydroxy, 21-cyano fused ring compound

14. A process according to claim 13, where the 1-aminomethylene group is temporarily protected to allow protection at the 18-hydroxy group, and the temporary protection is removed.

15. A process according to claim 13, wherein the C-18 hydroxy group is protected after formation of a 1-ester function.

16. A process according to claim 13, wherein the 1-aminomethylene group is converted to a 1-hydroxymethylene group and the 1-hydroxymethylne group is temporarily protected, to allow protection at the 18-hydroxy group, and the temporary protection is removed.

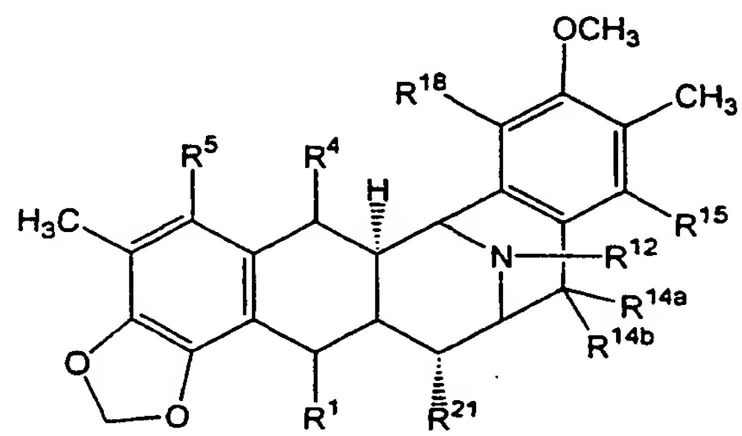
17. A process according to claim 1, wherein the 1-labile, 10-hydroxy, 18-protected hydroxy, di-6,8-en-5-one fused ring compound is prepared by steps starting from a 21-Nuc compound with a structure of formula (XIV):



where at least one ring *A* or *E* is quinolic, and where Nuc indicates the residue of a nucleophilic agent.

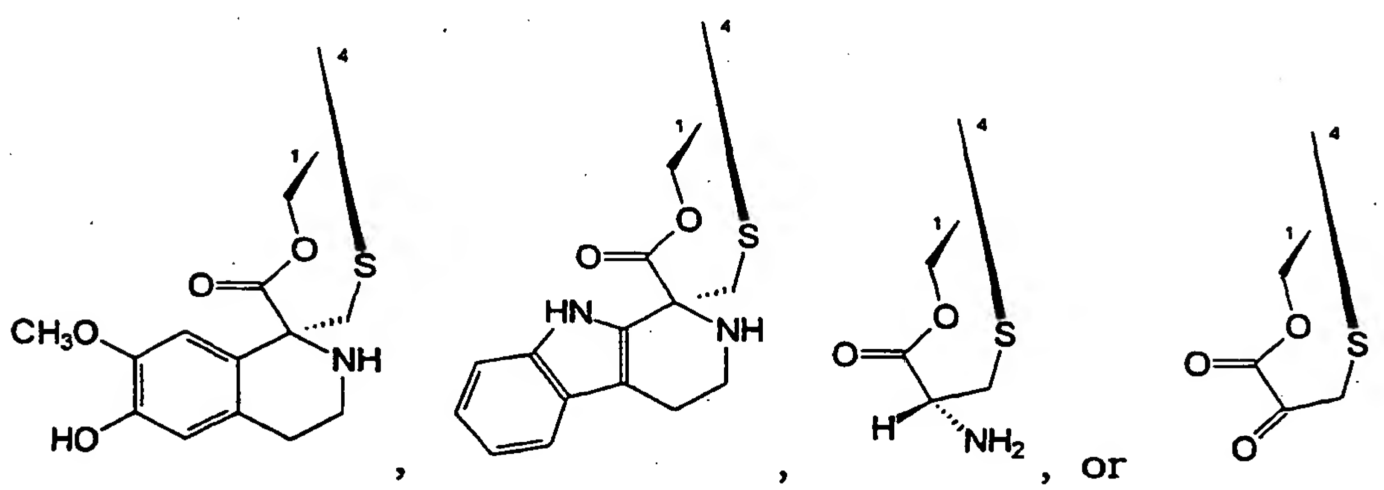
18. A process according to claim 17, wherein the compound of formula (XIV) is cyanosafracin B.

19. A process according to any preceding claim, wherein the product is of formula (XXIIb):



where:

R^1 and R^4 together form a group of formula (IV), (V), (VI) or (VII):



R^5 is -OH or a protected or derivatised version of such a group;

R^{14a} and R^{14b} are both -H or one is -H and the other is -OH or a protected or derivatised version of such a group, $-OCH_3$ or $-OCH_2CH_3$, or R^{14a} and R^{14b} together form a keto group;

R^{12} is $-NCH_3-$;

R^{15} is -OH or a protected or derivatised version of such a group; and

R^{18} is -OH or a protected or derivatised version of such a group.

20. A process according to claim 19, wherein R^5 is alkanoyloxy of 1 to 5 carbon atoms.

21. A process according to claim 20, wherein R^5 is acetyloxy.

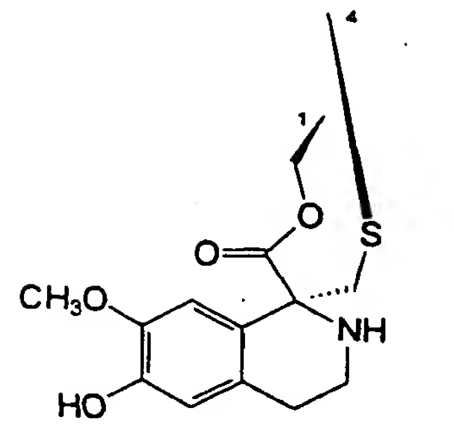
22. A process according to claim 19, 20 or 21, wherein R^{14a} and R^{14b} are hydrogen.

23. A process according to any of claims 19 to 22, wherein R^{15} is hydrogen.

24. A process according to any of claims 19 to 23, wherein R^{21} is -OH or -CN.

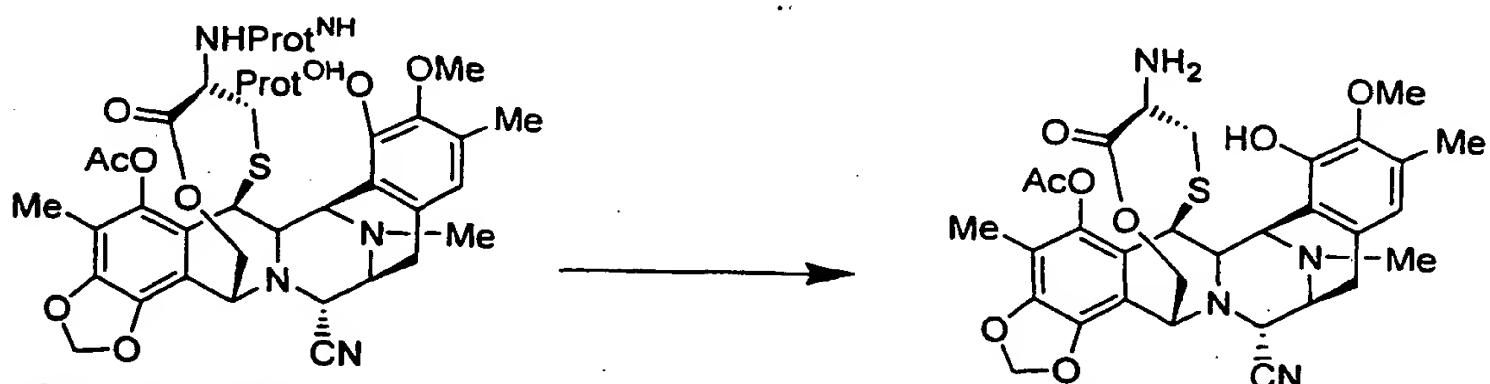
25. A process according to claim 11, wherein R^7 and R^8 together form a group $-O-CH_2-O-$.

26. A process according to any of claims 19 to 25, wherein R^1 and R^4 together form a group of formula (IV):



27. A process according to any preceding claim, wherein the ecteinascidin product is ecteinascidin 743.

28. A process step in the manufacture of an ecteinascidin comopund, the step comprising removing both protecting groups in a single step, in accordance with the following scheme:



where $Prot^{NH}$ is amino protecting group, and $Prot^{OH}$ is a hydroxy protecting group.

29. A process according to any of claims 1 to 27, which includes the process step according to claim 28.